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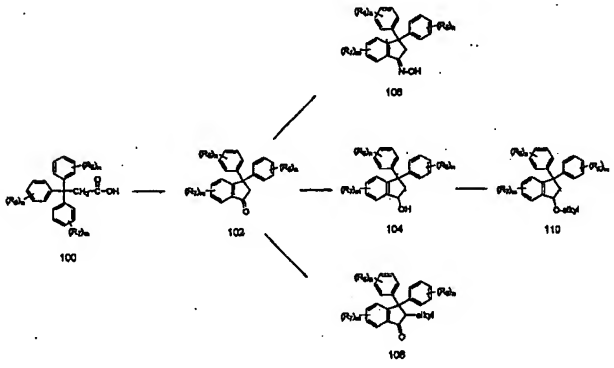

(57) Abstract

The present invention provides substituted 3,3-diphenyl indanone, indane and indole compounds, as well as analogues thereof which are specific, potent and safe inhibitors of the Ca^{2+}-activated potassium channel (Gardos channel) of erythrocytes, of mammalian cell proliferation and/or of secretagogue-stimulated transepithelial electrogenic chloride secretion in intestinal cells. The compounds can be used to reduce sickle erytrocyte dehydration and/or delay the occurrence of erythrocyte sickling or deformation *in situ* as a therapeutic approach towards the treatment or prevention of sickle cell disease. The compounds can also be used to inhibit mamalian cell proliferation *in situ* as a therapeutic approach towards the treatment or prevention of diseases characterized by abnormal cell proliferation. Furthermore, the compounds can also be used to inhibit chloride secretion in intestinal cells as a therapeutic approach towards the treatment of diarrhea and scours.

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USE OF SUBSTITUTED DIPHENYL INDANONE, INDANE AND INDOLE COMPOUNDS FOR THE TREATMENT OR PREVENTION OF SICKLE CELL DISEASE, INFLAMMATORY DISEASES CHARACTERIZED BY ABNORMAL CELL PROLIFERATION, DIARRHE AND SCOURS

Field of the Invention

The present invention relates to aromatic organic compounds which are specific, potent and safe inhibitors of the Ca^{2+}-activated potassium channel (Gardos channel) of erythrocytes, of mammalian cell proliferation, and/or of secretagogue-stimulated transepithelial electrogenic chloride secretion in intestinal cells. The compounds can be used to reduce sickle erythrocyte dehydration and/or delay the occurrence of erythrocyte sickling or deformation *in situ* as a therapeutic approach towards the treatment or prevention of sickle cell disease. The compounds can also be used to inhibit mammalian cell proliferation *in situ* as a therapeutic approach towards the treatment or prevention of diseases characterized by abnormal cell proliferation. Furthermore, the compounds can be used to inhibit chloride secretion as a therapeutic approach towards the treatment of diarrhea and scours.

Background of the Invention

Sickle cell disease has been recognized within West Africa for several centuries. Sickle cell anemia and the existence of sickle hemoglobin (Hb S) was the first genetic disease to be understood at the molecular level. It is recognized today as the morphological and clinical result of a glycine to valine substitution at the No. 6 position of the beta globin chain (Ingram, 1956, Nature 178:792-794). The origin of the amino acid change and of the disease state is the consequence of a single nucleotide substitution (Marotta *et al.*, 1977, J. Biol. Chem. 252:5040-5053).

The major source of morbidity and mortality of patients suffering from sickle cell disease is vascular occlusion caused by the sickled cells, which causes repeated episodes of pain in both acute and chronic form and also causes ongoing organ damage with the passage of time. It has long been recognized and accepted that the deformation and distortion of sickle cell erythrocytes upon complete deoxygenation is caused by polymerization and intracellular gelation of sickle hemoglobin, hemoglobin S (Hb S). The phenomenon is well reviewed and discussed by Eaton and Hofrichter, 1987, Blood 70:1245. The intracellular gelatin and polymerization of Hb S can occur at any time during erythrocyte's journey through the

vasculature. Thus, erythrocytes in patients with sickle cell disease containing no polymerized hemoglobin S may pass through the microcirculation and return to the lungs without sickling, may sickle in the veins or may sickle in the capillaries.

The probability of each of these events is determined by the delay time for intracellular gelation relative to the appropriate capillary transit time (Eaton *et al.*, 1976, Blood 47:621). In turn, the delay time is dependent upon the oxygenation state of the hemoglobin, with deoxygenation shortening the delay time. Thus, if it is thermodynamically impossible for intracellular gelation to take place, or if the delay time at venous oxygen pressures is longer than about 15 seconds, cell sickling will not occur. Alternatively, if the delay time is between about 1 and 15 seconds, the red cell will likely sickle in the veins. However, if the delay time is less than about 1 second, red cells will sickle within the capillaries.

For red cells that sickle within the capillaries, a number of possible consequent events exist, ranging from no effect on transit time, to transient occlusion of the capillary, to a more permanent blockage that may ultimately result in ischemia or infarction of the surrounding cells, and in destruction of the red cell.

It has long been recognized that the cytoplasm of the normal erythrocyte comprises approximately 70% water. Water crosses a normal erythrocyte membrane in milliseconds; however, the loss of cell water causes an exponential increase in cytoplasmic viscosity as the mean cell hemoglobin concentration (MCHC) rises above about 32 g/dl. Since cytoplasmic viscosity is a major determinate of erythrocyte deformability and sickling, the dehydration of the erythrocyte has substantial rheological and pathological consequences. Thus, the physiological mechanisms that maintain the water content of a normal erythrocytes and the pathological conditions that cause loss of water from erythrocytes in the blood circulation are critically important. Not surprisingly, regulation of erythrocyte dehydration has been recognized as an important therapeutic approach towards the treatment of sickle cell disease. Since cell water will follow any osmotic change in the intracellular concentration of ions, the maintenance of the red cell's potassium concentration is of particular importance (Stuart and Ellory, 1988, Brit J. Haematol. 69:1-4).

Many attempts and approaches to therapeutically treating dehydrated sickle cells (and thus decreasing polymerization of hemoglobin S by lowering the osmolality of plasma) have been tried with limited success, including the following approaches: intravenous infusion of

distilled water (Gye *et al.*, 1973, Am. J. Med. Sci. 266:267-277); administration of the antidiuretic hormone vasopressin together with a high fluid intake and salt restriction (Rosa *et al.*, 1980, M. Eng. J. Med. 303:1138-1143; Charache and Walker, 1981, Blood 58:892-896); the use of monensin to increase the cation content of the sickle cell (Clark *et al.*, 1982, J. Clin. Invest. 70:1074-1080; Fahim and Pressman, 1981, Life Sciences 29:1959-1966); intravenous administration of cetiedil citrate (Benjamin *et al.*, 1986, Blood 67:1442-1447; Berkowitz and Orringer, 1984, Am. J. Hematol. 17:217-223; Stuart *et al.*, 1987, J. Clin. Pathol. 40:1182-1186); and the use of oxpentifylline (Stuart *et al.*, 1987, J. Clin. Pathol. 40:1182-1186).

Another approach towards therapeutically treating dehydrated sickle cells involves the administration of imidazole, nitroimidazole and triazole antimycotic agents such as Clotrimazole (U.S. Patent No. 5,273,992 to Brugnara *et al.*). Clotrimazole, an imidazole-containing antimycotic agent, has been shown to be a specific, potent inhibitor of the Gardos channel of normal and sickle erythrocytes, and prevents Ca^{2+}-dependent dehydration of sickle cells both *in vitro* and *in vivo* (Brugnara *et al.*, 1993, J. Clin. Invest. 92:520-526; De Franceschi *et al.*, 1994, J. Clin. Invest. 93:1670-1676). When combined with a compound which stabilizes the oxyconformation of Hb S, Clotrimazole induces an additive reduction in the clogging rate of a micropore filter and may attenuate the formation of irreversibly sickled cells (Stuart *et al.*, 1994, J. Haematol. 86:820-823). Other compounds that contain a heteroaryl imidazole-like moiety believed to be useful in reducing sickle erythrocyte dehydration via Gardos channel inhibition include miconazole, econazole, butoconazole, oxiconazole and sulconazole. Each of these compounds is a known antimycotic. Other imidazole-containing compounds have been found to be incapable of inhibiting the Gardos channel and preventing loss of potassium.

As can be seen from the above discussion, reducing sickle erythrocyte dehydration *via* blockade of the Gardos channel is a powerful therapeutic approach towards the treatment and/or prevention of sickle cell disease. Compounds capable of inhibiting the Gardos channel as a means of reducing sickle cell dehydration are highly desirable, and are therefore an object of the present invention.

Cell proliferation is a normal part of mammalian existence, necessary for life itself. However, cell proliferation is not always desirable, and has recently been shown to be the root of many life-threatening diseases such as cancer, certain skin disorders, inflammatory

diseases, fibrotic conditions and arteriosclerotic conditions.

Cell proliferation is critically dependent on the regulated movement of ions across various cellular compartments, and is associated with the synthesis of DNA. Binding of specific polypeptide growth factors to specific receptors in growth-arrested cells triggers an array of early ionic signals that are critical in the cascade of mitogenic events eventually leading to DNA synthesis (Rozengurt, 1986, Science 234:161-164). These include (1) a rapid increase in cystolic Ca^{2+}, mostly due to rapid release of Ca^{2+} from intracellular stores; (2) capacitative Ca^{2+} influx in response to opening of ligand-bound and hyperpolarization-sensitive Ca^{2+} channels in the plasma membrane that contribute further to increased intracellular Ca^{2+} concentration (Tsien and Tsien, 1990, Annu. Rev. Cell Biol. 6:715-760; Peppelenbosch *et al.*, 1991, J. Biol. Chem. 266:19938-19944); and (3) activation of Ca^{2+}-dependent K^+ channels in the plasma membrane with increased K^+ conductance and membrane hyperpolarization (Magni *et al.*, 1991, J. Biol. Chem. 261:9321-9327). These mitogen-induced early ionic changes, considered critical events in the signal transduction pathways, are powerful therapeutic targets for inhibition of cell proliferation in normal and malignant cells.

One therapeutic approach towards the treatment of diseases characterized by unwanted or abnormal cell proliferation *via* alteration of the ionic fluxes associated with early mitogenic signals involves the administration of Clotrimazole. Clotrimazole has been shown to inhibit the Ca^{2+}-activated potassium channel of erythrocytes. In addition, Clotrimazole inhibits voltage- and ligand-stimulated Ca^{2+} influx mechanisms in nucleated cells (Villalobos *et al.*, 1992, FASEB J. 6:2742-2747; Montero *et al.*, 1991, Biochem. J. 277:73-79) and inhibits cell proliferation both *in vitro* and *in vivo* (Benzaquen *et al.*, 1995, Nature Medicine 1:534-540). Recently, Clotrimazole and other imidazole-containing antimycotic agents capable of inhibiting Ca^{2+}-activated potassium channels have been shown to be useful in the treatment of arteriosclerosis (U.S. Patent No. 5,358,959 to Halperin *et al.*), as well as other disorders characterized by unwanted or abnormal cell proliferation.

As can be seen from the above discussion, inhibiting mammalian cell proliferation *via* alteration of ionic fluxes associated with early mitogenic signals is a powerful therapeutic approach towards the treatment and/or prevention of diseases characterized by unwanted or abnormal cell proliferation. Compounds capable of inhibiting mammalian cell proliferation are highly desirable, and are therefore also an object of the present invention.

Acute and chronic diarrheas represent a major medical problem in many areas of the world. Diarrhea is both a significant factor in malnutrition and the leading cause of death (5,000,000 deaths/year) in children less than five years old. Secretory diarrheas are also a dangerous condition in patients of acquired immunodeficiency syndrome (AIDS) and chronic inflammatory bowel disease (IBD). 16 million travelers to developing countries from industrialized nations every year develop diarrhea, with the severity and number of cases of diarrhea varying depending on the country and area of travel. The major medical consequences of diarrheal diseases include dehydration, acidosis, death and impaired growth.

Diarrhea in barn animals and pets such as cows, pigs and horses, sheep, goats, cats and dogs, also known as scours, is a major cause of death in these animals. Diarrhea can result from any major transition, such as weaning or physical movement. One form of diarrhea is characterized by diarrhea in response to a bacterial or viral infection and generally occurs within the first few hours of the animal's life.

Although the major consequences of diarrheal diseases are very similar, there are numerous causes of diarrhea. Secretory and exudative diarrhea are primarily caused by bacterial or viral infections. The most common diarrheal causing bacteria is enterotoxogenic E-coli (ETEC) having the K99 pilus antigen. Common viral causes of diarrhea include rotavirus and coronavirus. Other infectious agents include cryptosporidium, giardia lamblia, and salmonella, among others.

The treatment for diarrhea depends on the patient and the infection source. Diarrhea which is found in travelers to industrialized nations (travelers diarrhea) frequently is caused by bacterial pathogens which are acquired through ingestion of fecally contaminated food and/or water. Approximately 50-75% of these cases are attributed to ETEC. Although traveler's diarrhea is painful, it is generally not life-threatening and often the symptoms last only 3-5 days. The symptoms include urgent diarrhea, abdominal cramps, nausea and fever. The most effective course of treatment for traveler's diarrhea is the administration of antibiotics in conjunction with oral rehydration. It has been shown that prophylactic administration of antibiotics drastically reduces the number of travelers experiencing symptoms of diarrhea. However, routine administration of antibiotics is not suggested as it may cause resistant strains of a bacteria to develop. Other treatment methods include administration of bismuth subsalicylate, often taken in the form of Pepto-Bismal, diphenoxylate and loperamide.

Diarrhea in AIDS patients is a very serious condition which causes wasting and may be an important factor in the decline of these patients. AIDS patients often develop diarrhea due to enteric infections which their immune system is not capable of fighting off, but AIDS patients may also develop diarrhea by AIDS enteropathy. AIDS enteropathy is a disorder characterized by diarrhea without the involvement of secondary infections. It is caused by the human immunodeficiency virus (HIV) infection of the small bowel mucosal cells and colonic mucosal cells. The most common infective agent causing diarrhea due to enteric infection in AIDS patients in cryptosporidium. The methods for treating diarrhea in AIDS patients include administration of antibiotics and administration of immunoglobulins or an immunoglobulin enriched fraction of bovine colostrum. Colostrum, which is the first milk produced by mammals after birthing is enriched with antibodies.

Acute diarrhea or scours, is a main cause of death in many newborn barn animals such as calves and pigs. Scours is often caused by ETEC with a K99 pilus antigen. Infection with the ETEC causes hypersecretion of fluid and electrolytes. Hypersecretion in turn causes dehydration and pH imbalance which may result in death of the newborn calf or pig.

Newborn barn animals are also susceptible to viral infectious agents causing scours. Infections with rotavirus and coronavirus are common in newborn calves and pigs. Rotavirus infection often occurs within 12 hours of birth. Symptoms of rotaviral infection include excretion of watery feces, dehydration and weakness. Coronavirus which causes a more severe illness in the newborn animals, has a higher mortality rate than rotaviral infection. Often, however, a young animal may be infected with more than one virus or with a combination of viral and bacterial microorganisms at one time. This dramatically increases the severity of the disease.

Generally the best protection for a newborn barn animal from viral or bacterial infection is the consumption of colostrum. If the mother animal has been exposed to these infectious agents then the colostrum will contain antibodies, which are often sufficient to protect the newborn from contracting the diseases. Sometimes, however, this is not sufficient and the animals need further protection. A common method of treatment includes administration of a concentrated colostrum solution or an immunoglobulin fraction isolated from a colostrum solution. This oral treatment may be combined with rehydration salts. Although these methods have improved the morbidity and mortality rate of newborn animals having scours, there still exists a need for more effective treatments.

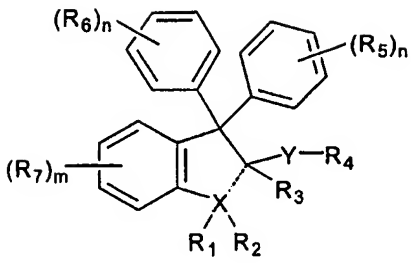
Certain imidazoles such as clotrimazole are agents which have been used both topically and systemically as antifungals. More recently, studies have identified other uses for such imidazoles. U.S. patent no. 5,273,992 revealed that these imidazoles regulate Ca^{++} activated K^+ channels in erythrocytes, and are thus useful in treating sickle cell anemia, which involves the inhibition of potassium transport. These imidazoles have also been found to be effective in inhibiting endothelial and/or vascular smooth muscle cell proliferation. The results of this finding are described in U.S. patent no. 5,358,959 and U.S. serial no. 08/018,840, which discloses using clotrimazole for treating atherosclerotic and angiogenic conditions, respectively. Nonimidazole metabolites and analogs of the foregoing compounds also have been described as useful in treating the foregoing conditions (see U.S. serials nos. 08/307,874 and 08/307,887).

Summary of the Invention

These and other objects are provided by the present invention, which in one aspect provides a class of organic compounds which are potent, selective and safe inhibitors of the Ca^{2+}-activated potassium channel (Gardos channel) of erythrocytes, of mammalian cell proliferation and/or of secretagogue-stimulated transepithelial electrogenic chloride secretion in intestinal cells. The compounds can be used to reduce sickle erythrocyte dehydration and/or delay the occurrence of erythrocyte sickling or deformation *in situ* as a therapeutic approach towards the treatment or prevention of sickle cell disease. The compounds can also be used to inhibit mammalian cell proliferation *in situ* as a therapeutic approach towards the treatment or prevention of diseases characterized by abnormal cell proliferation. Furthermore, the compounds can also be used to inhibit chloride secretion in intestinal cells as a therapeutic approach towards the treatment of diarrhea and scours. The compounds are generally substituted 3,3-diphenyl indanone, indane or (*3-H*) indole compounds, as well as analogues thereof.

In one illustrative embodiment, the compounds capable of inhibiting the Gardos channel, mammalian cell proliferation and/or secretagogue-stimulated transepithelial electrogenic chloride secretion in intestinal cells according to the invention, are compounds having the structural formula:

(I)



or pharmaceutically acceptable salts or hydrates thereof, wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', -NR'$_2$, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'$_2$ or -C(S)NR'$_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'$_2$, -ONR'$_2$, -SNR'$_2$, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -$CH(CN)_2$, -CH[C(O)R']$_2$, -CH[C(S)R']$_2$, -CH[C(O)OR']$_2$, -CH[C(S)OR']$_2$, -CH[C(O)SR']$_2$ and -CH[C(S)SR']$_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'$_2$, -OR', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group

consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', $-C(O)NR'_2$, $-C(S)NR'_2$ and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and

--- designates a single or double bond.

In a preferred embodiment of the invention, the chalcogens in the compounds of formula (I) are each oxygen.

In one embodiment, the substituents of the aromatic compounds of structural formula (I) are as follows: m is 0, 1, 2, 3 or 4; each n is independently 0, 1, 2, 3, 4 or 5; X is C or N; Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl; R_1 is absent, -OR, =O, =N-OR, -O-C(O)R, or when taken together with R_2 is a 3-8 membered oxirane or a substituted 3-8 membered oxirane; R_2 is absent or -H; R_3 is absent or -H; R_4 is -H, -OR', $-NR'_2$, -CN, $-NO_2$, (C_3-C_8) cycloalkyl, 3-8 membered oxiranyl, 5-8 membered dioxycycloalkyl, -C(O)R', -C(O)OR' or $-C(O)NR'_2$; each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', $-NR'_2$, $-ONR'_2$, $-NO_2$, -CN, -C(O)R', -C(O)OR', $-C(O)NR'_2$, -C(O)NR'(OR'), $-CH(CN)_2$, $-CH[C(O)R']_2$ and $-CH[C(O)OR']_2$; each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl; the oxirane substituents are each independently selected from the group consisting of -CN, $-NO_2$, $-NR'_2$, -OR', $-C(O)NR'_2$, -C(O)OR' and trihalomethyl; the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(O)OR', $-C(O)NR'_2$ and trihalomethyl; each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and/or --- designates a single or double bond.

In another preferred embodiment, the compounds are those of structural formula (I) wherein: m is 0 or 1; each n is independently 0 or 1; X is C or N; Y is absent, (C_1-C_3) alkyl, (C_1-C_3) alkenyl or (C_1-C_3) alkynyl; R_1 is absent -H, -OR, =O, $-NR_2$, =N-OR, -O-C(O)R, or when taken together with R_2 is 3-5 membered oxirane or 3-5 membered substituted oxirane; R_2 is absent or -H; R_3 is absent or -H; R_4 is -H, -OR, $-NR_2$, -CN, -C(O)OR, $-C(O)NR_2$ or 5-6 membered dioxoycycloalkyl; each R_5, R_6 and R_7 is independently selected from the group consisting of -R', -F, -Cl or -Br; each R is independently selected from the group consisting of -H, (C_1-C_3) alkyl, (C_1-C_3) alkenyl, (C_1-C_3) alkynyl, (C_5-C_{10}) aryl, substituted (C_5-C_{10}) aryl, (C_6-

C_{13}) alkaryl, substituted C_6-C_{13}) alkaryl; the oxirane substituent is -CN, -NO_2, -NR'_2, -OR' and trihalomethyl; the aryl and alkaryl substituents are each independently selected from the group consisting of -F, -Cl, -Br, -CN, -NO_2, -NR'_2, -C(O)R', -C(O)OR' and trihalomethyl; R' is -H, (C_1-C_3) alkyl, (C_1-C_3) alkenyl or (C_1-C_3) alkynyl; and/or --- is a single or double bond.

In still another preferred embodiment, the compounds are those of structural formula (I) wherein: m is 0, 1, 2, 3 or 4; each n is independently 0, 1, 2, 3, 4 or 5; X is C or N; Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl; R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl; R_2 is absent or -H; R_3 is absent or -H; R_4 is -H, -OR', -SR', -NR'_2, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -$C(O)NR'_2$ or -$C(S)NR'_2$; each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'_2, -ONR'_2, -SNR'_2, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -$C(O)NR'_2$, -$C(S)NR'_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -$CH(CN)_2$, -$CH[C(O)R']_2$, -$CH[C(S)R']_2$, -$CH[C(O)OR']_2$, -$CH[C(S)OR']_2$, -$CH[C(O)SR']_2$ and -$CH[C(S)SR']_2$; each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl; the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -$C(O)NR'_2$, -$C(S)NR'_2$, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl; the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -$C(O)NR'_2$, -$C(S)NR'_2$ and trihalomethyl; each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; --- designates a single or double bond; and wherein when X is C and R_1 is =O, =S or -OR', at least one of R_5, R_6 or R_7 is other than -R', preferably other than -H, or Y is present or R_4 is other than -H; and when X is N, --- is a double bond and R_1, R_2, R_3 and Y are absent, R_4 is other than -NR'_2, preferably other than -NH_2.

In still another preferred embodiment, the compounds are those of structural formula (I) wherein: m is 0, 1, 2, 3 or 4; each n is independently 0, 1, 2, 3, 4 or 5; X is C; Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl; R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8

membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl; R_2 is absent or -H; R_3 is absent or -H; R_4 is -H, -OR', -SR', -NR'_2, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -$C(O)NR'_2$ or -$C(S)NR'_2$; each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'_2, -ONR'_2, -SNR'_2, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -$C(O)NR'_2$, -$C(S)NR'_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -$CH(CN)_2$, -$CH[C(O)R']_2$, -$CH[C(S)R']_2$, -$CH[C(O)OR']_2$, -$CH[C(S)OR']_2$, -$CH[C(O)SR']_2$ and -$CH[C(S)SR']_2$; each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl; the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -$C(O)NR'_2$, -$C(S)NR'_2$, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl; the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -$C(O)NR'_2$, -$C(S)NR'_2$ and trihalomethyl; each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; --- designates a single or double bond; and wherein when R_1 is =O or -OH, at least one of R_5, R_6 or R_7 is other than -R', preferably other than -H, or Y is present or R_4 is other than -H.

In still another aspect, the invention provides a method for reducing sickle erythrocyte dehydration and/or delaying the occurrence of erythrocyte sickling or deformation *in situ*. The method involves contacting a sickle erythrocyte *in situ* with an amount of at least one compound according to the invention, or a pharmaceutical composition thereof, effective to reduce sickle erythrocyte dehydration and/or delay the occurrence of erythrocyte sickling or deformation. In a preferred embodiment, the sickle cell dehydration is reduced and erythrocyte deformation is delayed in a sickle erythrocyte that is within the microcirculation vasculature of a subject, thereby preventing or reducing the vaso-occlusion and consequent adverse effects that are commonly caused by sickled cells.

In still another aspect, the invention provides a method for the treatment and/or prevention of sickle cell disease in a subject, such as a human. The method involves administering a prophylactically or therapeutically effective amount of at least one compound according to the invention, or a pharmaceutical composition thereof, to a patient suffering from sickle cell disease. The patient may be suffering from either acute sickle crisis or

chronic sickle cell episodes.

In one aspect of the invention a method is provided for inhibiting unwanted cellular proliferation associated with an inflammatory disease. The method includes the step of contacting a cell the proliferation of which contributes to inflammation *in situ* with an amount of a compound having the above described formula (I) effective to inhibit proliferation of the cell. In one embodiment the method of administration is selected from the group consisting of oral, parenteral, intravenous, subcutaneous, transdermal and transmucosal for a living human. In one embodiment the mammalian cell is a fibrotic cell or a lymphocyte.

According to another aspect of the invention a method is provided for treating or preventing an inflammatory disease. The method includes the step of administering to a subject in need of such treatment a therapeutically effective amount of a compound of the above-described formula (I). In one embodiment the inflammatory disease is diarrhea. Preferably the diarrhea is caused by inflammatory bowel disease. In another embodiment the inflammatory disease is an autoimmune disease. In other embodiments the inflammatory disease is selected from the group consisting of proliferative glomerulonephritis; lupus erythematosus; scleroderma; temporal arteritis; thromboangiitis obliterans; mucocutaneous lymph node syndrome; asthma; host versus graft; inflammatory bowel disease; multiple sclerosis; rheumatoid arthritis; thyroiditis; Grave's disease; antigen-induced airway hyperactivity; pulmonary eosinophilia; Guillain-Barre syndrome; allergic rhinitis; myasthenia gravis; human T-lymphotrophic virus type 1-associated myelopathy; herpes simplex encephalitis; inflammatory myopathies; atherosclerosis; and Goodpasture's syndrome. In certain embodiments the administration is parenteral or per oral.

In yet another aspect, the invention provides a method for inhibiting mammalian cell proliferation *in situ.* Preferably, the mammalian cell proliferation is not associated with a proliferative disease selected from the group consisting of cancer, actinic keratosis, and Kaposi's sarcoma. The method involves contacting a mammalian cell *in situ* with an amount of at least one compound according to the invention, or a pharmaceutical composition thereof, effective to inhibit cell proliferation. The compound or composition may act either cytostatically, cytotoxically or a by a combination of both mechanisms to inhibit proliferation. Mammalian cells in this manner include vascular smooth muscle cells, fibroblasts and endothelial cells.

In still another aspect, the invention provides a method for treating and/or preventing

unwanted or abnormal cell proliferation in a subject, such as a human. Preferably, the unwanted or abnormal cell proliferation is not associated with a proliferative disease selected from the group consisting of cancer, actinic keratosis, and Kaposi's sarcoma. In the method, at least one compound according to the invention, or a pharmaceutical composition thereof, is administered to a subject in need of such treatment in an amount effective to inhibit the unwanted or abnormal mammalian cell proliferation. The compound and/or composition may be applied locally to the proliferating cells, or may be administered to the subject systemically. Preferably, the compound and/or composition is administered to a subject that has a disorder characterized by unwanted or abnormal cell proliferation, and preferably the unwanted or abnormal cell proliferation is not associated with a proliferative disease selected from the group consisting of cancer, actinic keratosis, and Kaposi's sarcoma. Such disorders include, but are not limited to, non-cancerous angiogenic conditions or arteriosclerosis.

In yet another aspect, the invention provides a method for the treatment and/or prevention of diseases that are characterized by unwanted and/or abnormal mammalian cell proliferation. Preferably, the unwanted or abnormal cell proliferation is not associated with a proliferative disease selected from the group consisting of cancer, actinic keratosis, and Kaposi's sarcoma. The method involves administering a prophylactically or therapeutically effective amount of at least one compound according to the invention, or a pharmaceutical composition thereof, to a subject in need of such treatment. Diseases that are characterized by abnormal mammalian cell proliferation which can be treated or prevented by way of the methods of the invention include, but are not limited to, blood vessel proliferative disorders, fibrotic disorders and arteriosclerotic conditions.

According to another aspect of the invention, a method for treating diarrhea of diverse etiology is provided. The method involves administering to a subject who is in need of such treatment, an aromatic compound of the invention in an amount effective to inhibit the diarrhea. Preferably the compound is administered orally in conjunction with oral rehydration fluids. The aromatic compounds useful in the invention are generally substituted 3,3-diphenyl indanone, indane or (*3-H*) indole compounds, or analogues thereof.

In one embodiment of the invention the foregoing aromatic compounds may be administered in combination with other non-formula (I) anti-diarrheal agents. In another embodiment the aromatic compounds may be administered in combination with other non-formula (I) anti-scours agents.

According to one embodiment of the invention the subject in need of such treatment is a subject who has symptoms of diarrhea or scours. In another embodiment of the invention, the subject in need of such treatment is a subject at risk of developing diarrhea or scours.

In general diarrhea is a secretory disorder, which is caused by at least one of several mechanisms. In one embodiment the diarrhea is an exudative form of diarrhea; In one embodiment the diarrhea is a nonexudative form of diarrhea; In another embodiment the diarrhea is a decreased absorption form of diarrhea; In another embodiment the diarrhea is a non-decreased absorption form of diarrhea; In yet another embodiment the diarrhea is a secretory form of diarrhea. In yet another embodiment the diarrhea is a nonsecretory form of diarrhea. In still another embodiment the diarrhea is a noninflammatory form of diarrhea.

In another aspect, the present invention provides pharmaceutical compositions comprising one or more compounds according to the invention in admixture with a pharmaceutically acceptable carrier, excipient or diluent. Such a preparation can be administered in the methods of the invention.

According to another aspect of the invention, pharmaceutical preparations are provided, comprising one or more of the aromatic compounds of the invention in admixture with a pharmaceutically acceptable carrier, excipient or diluent, wherein the aromatic compound(s) of the invention is (are) in an amount effective for treating: (i) unwanted or abnormal cell proliferation, preferably not a proliferative disease selected from the group consisting of cancer, actinic keratosis, and Kaposi's sarcoma; (ii) an inflammatory disease; (iii) sickle cell disease; and (iv) diarrhea or scours. In one embodiment, the aromatic compounds useful according to the invention have the general formula (I) provided above. In certain other embodiments, the pharmaceutical preparations include the aromatic compounds of the invention together with a non-formula (I) agent selected from the group consisting of an anti-proliferative agent; (ii) an anti-inflammatory agent; (iii) anti-sickle cell agent; and (iv) an anti-diarrhea or anti-scours agent.

According to another aspect, the use of aromatic compounds of the invention in the manufacture of medicaments is provided. The medicaments are useful for treating: (i) unwanted or abnormal cell proliferation, preferably not a proliferative disease that includes cancer, actinic keratosis, and Kaposi's sarcoma; (ii) an inflammatory disease; (iii) sickle cell disease; and (iv) diarrhea or scours.

According to another aspect of the invention, pharmaceutical preparations are provided. These pharmaceutical preparations include the aromatic compounds of the invention together with an anti-diarrheal agent. In one embodiment, the aromatic compounds useful according to the invention have the general formula (I) provided above. In other embodiments the aromatic compounds useful according to the invention are the preferred compounds described above. Preferably the pharmaceutical composition of the invention may be administered orally.

The invention also provides the aromatic compounds of the invention in the manufacture of a medicament for the treatment of diarrhea or scours. In one embodiment, the aromatic compounds useful according to the invention have the general formula (I) provided above. In other embodiments the aromatic compounds useful according to the invention are the preferred compounds described above.

According to another aspect of the invention, veterinary preparations are provided. These veterinary preparations include the aromatic compounds useful according to the invention together with an anti-scours preparation. In one embodiment, the aromatic compounds useful according to the invention have the general formula (I) provided above. In other embodiments the aromatic compounds useful according to the invention are the preferred compounds described above.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention.

Brief Description of the Drawings

FIG. 1 is a general reaction scheme for synthesizing certain compounds according to the invention;

FIG. 2 is a general reaction scheme for synthesizing certain compounds according to the invention;

FIG. 3 is a bar graph depicting the effect of clotrimazole in the inhibition of cAMP and Ca^{++} dependent Cl^- secretion in T84 cells; and

FIG. 4 is a graph showing the effect of clotrimazole on the inhibition of base line and

Ca^{++} - stimulated ^{86}Rb efflux from T84 monolayers.

Detailed Description of the Invention

As discussed in the Background section, blockade of sickle dehydration *via* inhibition of the Gardos channel is a powerful therapeutic approach towards the treatment and/or prevention of sickle cell disease. *In vitro* studies have shown that Clotrimazole, an imidazole-containing antimycotic agent, blocks Ca^{2+}-activated K^+ transport and cell dehydration in sickle erythrocytes (Brugnara *et al.*, 1993, J. Clin. Invest. 92:520-526). Studies in a transgenic mouse model for sickle cell disease (SAD mouse, Trudel *et al.*, 1991, EMBO J. 11:3157-3165) show that oral administration of Clotrimazole leads to inhibition of the red cell Gardos channel, increased red cell K^+ content, a decreased mean cell hemoglobin concentration (MCHC) and decreased cell density (De Franceschi *et al.*, 1994, J. Clin. Invest. 93:1670-1676). Moreover, therapy with oral Clotrimazole induces inhibition of the Gardos channel and reduces erythrocyte dehydration in patients with sickle cell disease (Brugnara *et al.*, 1996, J. Clin. Invest. 97:1227-1234). Other antimycotic agents which inhibit the Gardos channel *in vitro* include miconazole, econazole, butoconazole, oxiconazole and sulconazole (U.S. Patent No. 5,273,992 to Brugnara *et al.*). All of these compounds contain an imidazole-like ring, *i.e.*, a heteroaryl ring containing two or more nitrogens.

Also as discussed in the Background section, the modulation of early ionic mitogenic signals and inhibition of cell proliferation are powerful therapeutic approaches towards the treatment and/or prevention of disorders characterized by abnormal cell proliferation. It has been shown that Clotrimazole, in addition to inhibiting the Gardos channel of erythrocytes, also modulates ionic mitogenic signals and inhibits cell proliferation both *in vitro* and *in vivo*.

For example, Clotrimazole inhibits the rate of cell proliferation of normal and cancer cell lines in a reversible and dose-dependent manner *in vitro* (Benzaquen *et al.*, 1995 Nature Medicine 1:534-540). Clotrimazole also depletes the intracellular Ca^{2+} stores and prevents the rise in cystolic Ca^{2+} that normally follows mitogenic stimulation. Moreover, in mice with severe combined immunodeficiency disease (SCID) and inoculated with MM-RU human melanoma cells, daily administration of Clotrimazole resulted in a significant reduction in the number of lung metastases observed (Benzaquen *et al.*, *supra*).

It has now been discovered that substituted 3,3-diphenyl indanone, indane and (*3-H*) indole compounds, as well as analogues of these classes of compounds, also inhibit the

Gardos channel of erythrocytes, mammalian cell proliferation and/or Cl^- secretion from intestinal cells. Thus, in one aspect, the present invention provides a new class of organic compounds that are capable of inhibiting the Gardos channel of erythrocytes, mammalian cell proliferation, particularly mitogen-induced cell proliferation, and/or Cl^- secretion from intestinal cells.

The activities of these compounds are quite surprising. Significantly, the compounds of the invention do not contain an imidazole or imidazole-like moiety. The imidazole or imidazole-like moiety is well-recognized as the essential functionality underlying the antimycotic and other biological activities of Clotrimazole and the other above-mentioned anti-mycotic agents. Thus, the substituted 3,3-diphenyl indanone, indane or (*3-H*) indole compounds and analogues of the invention provide an entirely new class of compounds capable of effecting inhibition the Ca^{2+}-activated potassium channel (Gardos channel) of erythrocytes, particularly sickle erythrocytes, mammalian cell proliferation, particularly mitogen-induced cell proliferation, and/or Cl^- secretion from intestinal cells.

In another aspect, the invention provides a method of reducing sickle cell dehydration and/or delaying the occurrence of erythrocyte sickling *in situ* as a therapeutic approach towards the treatment of sickle cell disease. In its broadest sense, the method involves only a single step -- the administration of at least one pharmacologically active compound of the invention, or a composition thereof, to a sickle erythrocyte *in situ* in an amount effective to reduce dehydration and/or delay the occurrence of cell sickling or deformation.

While not intending to be bound by any particular theory, it is believed that administration of the active compounds described herein in appropriate amounts to sickle erythrocytes *in situ* causes nearly complete inhibition of the Gardos channel of sickle cells, thereby reducing the dehydration of sickle cells and/or delaying the occurrence of cell sickling or deformation. In a preferred embodiment, the dehydration of a sickle cell is reduced and/or the occurrence of sickling is delayed in a sickle cell that is within the microcirculation vasculature of the subject, thereby reducing or eliminating the vaso-occlusion that is commonly caused by sickled cells.

Based in part on the surmised importance of the Gardos channel as a therapeutic target in the treatment of sickle cell disease, the invention is also directed to methods of treating or preventing sickle cell disease. In the method, an effective amount of one or more compounds according to the invention, or a pharmaceutical composition thereof, is administered to a

patient suffering from sickle cell disease. The methods may be used to treat sickle cell disease prophylactically to decrease intracellular Hb S concentration and/or polymerization, and thus diminish the time and duration of red cell sickling and vaso-occlusion in the blood circulation. The methods may also be used therapeutically in patients with acute sickle cell crisis, and in patients suffering chronic sickle cell episodes to control both the frequency and duration of the crises.

The compounds of the invention are also potent, specific inhibitors of mammalian cell proliferation. Thus, in another aspect, the invention provides methods of inhibiting mammalian cell proliferation as a therapeutic approach towards the treatment or prevention of diseases characterized by unwanted or abnormal cell proliferation. In its broadest sense, the method involves only a single step-- the administration of an effective amount of at least one pharmacologically active compound according to the invention to a mammalian cell *in situ*. The compound may act cytostatically, cytotoxically, or by a combination of both mechanisms to inhibit cell proliferation. Mammalian cells treatable in this manner include vascular smooth muscle cells, fibroblasts, endothelial cells, various pre-cancer cells and various cancer cells. In a preferred embodiment, cell proliferation is inhibited in a subject suffering from a disorder that is characterized by unwanted or abnormal cell proliferation. Such diseases are described more fully below.

Based in part on the surmised role of mammalian cell proliferation in certain diseases, the invention is also directed to methods of treating or preventing diseases characterized by abnormal cell proliferation. In the method, an effective amount of at least one compound according to the invention, or a pharmaceutical composition thereof, is administered to a patient suffering from a disorder that is characterized by abnormal cell proliferation. While not intending to be bound by any particular theory, it is believed that administration of an appropriate amount of a compound according to the invention to a subject inhibits cell proliferation by altering the ionic fluxes associated with early mitogenic signals. Such alteration of ionic fluxes is thought to be due to the ability of the compounds of the invention to inhibit potassium channels of cells, particularly Ca^{2+}-activated potassium channels. The method can be used prophylactically to prevent unwanted or abnormal cell proliferation, or may be used therapeutically to reduce or arrest proliferation of abnormally proliferating cells. The compound, or a pharmaceutical formulation thereof, can be applied locally to proliferating cells to arrest or inhibit proliferation at a desired time, or may be administered to

a subject systemically to arrest or inhibit cell proliferation.

Diseases which are characterized by abnormal cell proliferation that can be treated or prevented by means of the present invention include blood vessel proliferative disorders, fibrotic disorders, arteriosclerotic disorders and various cancers.

Blood vessel proliferation disorders refer to angiogenic and vasculogenic disorders generally resulting in abnormal proliferation of blood vessels. The formation and spreading of blood vessels, or vasculogenesis and angiogenesis, respectively, play important roles in a variety of physiological processes such as embryonic development, corpus luteum formation, wound healing and organ regeneration. They also play a pivotal role in cancer development. Other examples of blood vessel proliferative disorders include arteritis, where new capillary blood vessels invade the joint and destroy cartilage and ocular diseases such as diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness and neovascular glaucoma.

Another example of abnormal neovascularization is that associated with solid tumors. It is now established that unrestricted growth of tumors is dependent upon angiogenesis and that induction of angiogenesis by liberation of angiogenic factors can be an important step in carcinogenesis. For example, basic fibroblast growth factor (bFGF) is liberated by several cancer cells and plays a crucial role in cancer angiogenesis. The demonstration that certain animal tumors regress when angiogenesis is inhibited has provided the most compelling evidence for the role of angiogenesis in tumor growth. Other cancers that are associated with neovascularization include hemangioendotheliomas, hemangiomas and Kaposi's sarcoma.

Proliferation of endothelial and vascular smooth muscle cells is the main feature of neovascularization. The invention is useful in inhibiting such proliferation, and therefore in inhibiting or arresting altogether the progression of the angiogenic condition which depends in whole or in part upon such neovascularization. The invention is particularly useful when the condition has an additional element of endothelial or vascular smooth muscle cell proliferation that is not necessarily associated with neovascularization. For example, psoriasis may additionally involve endothelial cell proliferation that is independent of the endothelial cell proliferation associated with neovascularization. Likewise, a solid tumor which requires neovascularization for continued growth may also be a tumor of endothelial or vascular smooth muscle cells. In this case, growth of the tumor cells themselves, as well as the neovascularization, is inhibited by the compounds described herein.

The invention is also useful for the treatment of fibrotic disorders such as fibrosis and other medical complications of fibrosis which result in whole or in part from the proliferation of fibroblasts. Medical conditions involving fibrosis (other than atherosclerosis, discussed below) include undesirable tissue adhesion resulting from surgery or injury.

Other cell proliferative disorders which can be treated by means of the invention include arteriosclerotic conditions. Arteriosclerosis is a term used to describe a thickening and hardening of the arterial wall. An arteriosclerotic condition as used herein means classical atherosclerosis, accelerated atherosclerosis, atherosclerotic lesions and any other arteriosclerotic conditions characterized by undesirable endothelial and/or vascular smooth muscle cell proliferation, including vascular complications of diabetes.

Proliferation of vascular smooth muscle cells is a main pathological feature in classical atherosclerosis. It is believed that liberation of growth factors from endothelial cells stimulates the proliferation of subintimal smooth muscle which, in turn, reduces the caliber and finally obstructs the artery. The invention is useful in inhibiting such proliferation, and therefore in delaying the onset of, inhibiting the progression of, or even halting the progression of such proliferation and the associated atherosclerotic condition.

Proliferation of vascular smooth muscle cells produces accelerated atherosclerosis, which is the main reason for failure of heart transplants that are not rejected. This proliferation is also believed to be mediated by growth factors, and can ultimately result in obstruction of the coronary arteries. The invention is useful in inhibiting such obstruction and reducing the risk of, or even preventing, such failures.

Vascular injury can also result in endothelial and vascular smooth muscle cell proliferation. The injury can be caused by any number of traumatic events or interventions, including vascular surgery and balloon angioplasty. Restenosis is the main complication of successful balloon angioplasty of the coronary arteries. It is believed to be caused by the release of growth factors as a result of mechanical injury to the endothelial cells lining the coronary arteries. Thus, by inhibiting unwanted endothelial and smooth muscle cell proliferation, the compounds described herein can be used to delay, or even avoid, the onset of restenosis.

Other atherosclerotic conditions which can be treated or prevented by means of the present invention include diseases of the arterial walls that involve proliferation of endothelial and/or vascular smooth muscle cells, such as complications of diabetes, diabetic

glomerulosclerosis and diabetic retinopathy.

The compounds described herein are also useful in treating or preventing various types of cancers. Cancers which can be treated by means of the present invention include, but are not limited to, biliary tract cancer; brain cancer, including glioblastomas and medulloblastomas; breast cancer; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; hematological neoplasms, including acute and chronic lymphocytic and myelogenous leukemia, multiple myeloma, AIDS associated leukemias and adult T-cell leukemia lymphoma; intraepithelial neoplasms, including Bowen's disease and Paget's disease; liver cancer; lung cancer; lymphomas, including Hodgkin's disease and lymphocytic lymphomas; neuroblastomas; oral cancer, including squamous cell carcinoma; ovarian cancer, including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreas cancer; prostate cancer; rectal cancer; sarcomas, including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma and osteosarcoma; skin cancer, including melanoma, Kaposi's sarcoma, basocellular cancer and squamous cell cancer; testicular cancer, including germinal tumors (seminoma, non-seminoma (teratomas, choriocarcinomas)), stromal tumors and germ cell tumors; thyroid cancer, including thyroid adenocarcinoma and medullar carcinoma; and renal cancer including adenocarcinoma and Wilms tumor.

The compounds of the invention are useful with hormone dependent and also with nonhormone dependent cancers. They also are useful with prostate and nonprostate cancers and with breast and nonbreast cancers. They further are useful with multidrug resistant strains of cancer.

In addition to the particular disorders enumerated above, the invention is also useful in treating or preventing dermatological diseases including keloids, hypertrophic scars, seborrheic dermatosis, papilloma virus infection (*e.g.*, producing verruca vulgaris, verruca plantaris, verruca plan, condylomata, etc.), and eczema and epithelial precancerous lesions such as actinic keratosis. It also is useful with pathologies mediated by growth factors such as uterine leiomyomas.

In addition to the particular disorders enumerated above, the invention is particularly useful in treating or preventing inflammatory diseases associated with cellular proliferation. An "inflammatory disease associated with cellular proliferation" as used herein is a disease in which lymphoproliferation contributes to tissue or organ damage leading to disease. For

instance, excessive T cell proliferation at the site of a tissue or organ will cause damage to the tissue or organ. Inflammatory disease are well known in the art and have been described extensively in medical textbooks (See, e.g., Harrison's Principles of Experimental Medicine, 13th Edition, McGraw-Hill, Inc., N.Y.).

Inflammatory diseases associated with cellular proliferation include but are not limited to proliferative glomerulonephritis; lupus erythematosus; scleroderma; temporal arteritis; thromboangiitis obliterans; mucocutaneous lymph node syndrome; asthma; host versus graft; inflammatory bowel disease; multiple sclerosis; rheumatoid arthritis; thyroiditis; Grave's disease; antigen-induced airway hyperactivity; pulmonary eosinophilia; Guillain-Barre syndrome; allergic rhinitis; myasthenia gravis; human T-lymphotrophic virus type 1-associated myelopathy; herpes simplex encephalitis; inflammatory myopathies; atherosclerosis; and Goodpasture's syndrome. Some examples of inflammatory diseases associated with cellular proliferation as well as animal models for testing and developing the compounds are set forth in Table 1 below.

Table 1

Disease	Proliferating Cells	Reference	Animal Model	Reference
Asthma	T cells	Hogg 1997 APMIS 100:105(10):735-45	Airway inflammation and hyperresponsiveness in Ovalbumin-sensitized mice or guinea pigs.	Henderson et al. 1997 J Clin Invest 100(12):3083-3092.
Glomerulonephritis	Mesangial (glomerular) cells	Nitta et al. 1998 Eur J Pharmacol 344:107-110	NZB/NZW crossed mice develop glomerular disease and lupus-like syndrome.	Clynes et al. 1998 Science 279(5353): 1052-54.
Host versus Graft	T cells B cells	Schorlemmer et al. 1997 Int J Tissue React 19:157-61. Sedgwick et al. 1998 J Immunol 160:5320-30.	Renal allograft rejection in mice.	Lazarivuts et al. 1996 Nature 380(6576) 717-720.
Inflammatory Bowel Disease	Epithelial cells	Bajaj-Elliott et al. 1997 Am J. Pathol. 151:1469-76.	Trinitrobenzene sulphonic acid induced bowel inflammation in rats.	Boughton-Smith et al. 1988 Br J Pharmacol 94:65-72.
Systemic Lupus Erythematosis	Glomerular cells Lymphocytes	Kodera et al. 1997 Am J Nephol 17:466-70. Akashi et al. 1998 Immunology 93:238-48	NZB/NZW crossed mice develop glomerular disease and lupus-like syndrome.	Peng et al. 1996 Mol Biol Rep 23(3-4):247-51.

Multiple Sclerosis	T cells	Constantinesecu et al. 1998 Immunol Res 17(1-2):217-27.	Experimental allergic encephalomyelitis.	Drescher et al. 1998 J Clin Invest 101(8):1765-74.
Rheumatoid Arthritis	T cells Synovial cells	Ceponis et al. 1998 Br J Rheumatol 37(2):170-8	Rat adjuvant arthritis assay	Anderson et al. 1996 J Clin Invest 97(11):2672-9.
Thyroiditis	T cells and Epithelial cells	Rose et al. 1997 Crit Rev Immunol 17:511-7. Schumm-Draeger et al. 1996 Verh Dtsch Ges Pathol 80:297-301.	HLA transgenic mice immunized with thyroglobulin.	Taneja et al. 1998 J Clin Investig 101(5):921-6.
Grave's Disease	Thyroid cells	DiPaola et al. 1997 J Clin Endocrinol Metab 82:670-3.	Thiouracil-fed rats.	Viglietto et al. 1997 Oncogene 15:2687-98.

Disease	Proliferating Cells	Reference	Model
Antigen-induced airway hyperactivity	T cells	Wolyniec et al. 1998 Am J Respir Cell Mol Biol 18:777-85	
Pulmonary eosinophilia	T cells	Wolyniec et al. 1998 Am J Respir Cell Mol Biol 18:777-85	
Guillain-Barre Syndrome (inflammatory demyelinating disease)	T cells	Hartung et al. 1991 Ann Neurol. 30:48-53	Experimental autoimmune neuritis (immunization with PNS myelin and Freunds complete adjuvant)
Giant cell arteritis (a form of systemic vasculitis) Inflammation of large arteries	T cells	Brack et al. 1997 Mol Med 3:530-43	
Allergic Rhinitis	T cells	Baraniuk et al. 1997 J Allergy Clin Immunol 99:S763-72	
Myasthenia gravis	T cells	Hartung et al. 1991 Ann Neurol 30:48-53	
Human T-lymphotropic virus type 1 - associated myelopathy	T cells	Nakamura et al. 1996 Intern Mede 35:195-99	
Herpes simplex encephalitis	T cells	Hartung et al. 1991 Ann Neurol 30:48-53	
Inflammatory myopathies (ie. Polymyositis, dermatomyocitis)	T cells	Hartung et al. 1991 Ann Neurol 30:48-53. Lindberg et al. 1995 Scand J Immunol 41:421-26	

Artherosclerosis	T cells	Rosenfeld et al. 1996 Diabetes Res Clin Pract 30 suppl.: 1-11	
Goodpasture's syndrome	Macrophages	Lan et al. 1995 Am J Pathol 147:1214-20	

The compounds and methods of the invention provide myriad advantages over agents and methods commonly used to treat cell proliferative disorders. For example, many of the compounds of the invention are more potent than Clotrimazole in *in vitro* tests, and therefore may provide consequential therapeutic advantages in clinical settings.

Most significantly, the compounds of the invention have reduced toxicity as compared with these other agents. For Clotrimazole, it is well-known that the imidazole moiety is responsible for inhibiting a wide range of cytochrome P-450 isozyme catalyzed reactions, which constitutes their main toxicological effects (Pappas and Franklin, 1993, Toxicology 80:27-35; Matsuura *et al.*, 1991, Biochemical Pharmacology 41:1949-1956). Analogues and metabolites of Clotrimazole do not induce cytochrome P-450 (Matsuura *et al.*, 1991, Biochemical Pharmacology 41:1949-1956), and therefore do not share Clotrimazole's toxicity.

The invention in another aspect also involves methods and products for reducing the symptoms of diarrhea or preventing diarrhea in a subject at risk for developing diarrhea, using the compounds of the invention. The aromatic compounds useful according to the invention may be provided in a pharmaceutical preparation or a veterinary preparation. The aromatic compounds of the invention are also useful in a method for treating diarrhea and scours as well as a method for preventing diarrhea and scours.

Diarrhea, as used herein, indicates a medical syndrome which is characterized by the symptoms of diarrhea or scours. In general, diarrhea is a disorder resulting in a secretory imbalance. For purposes of this patent application diarrhea is divided into three categories based on the underlying mechanism: exudative, decreased absorption, and secretory and the term diarrhea as used herein encompasses each of these categories. Exudative diarrheas result from inflammatory processes leading to impaired colonic absorption, and outpouring of cells and colloid caused by such disorders as ulcerative colitis, shigellosis, and amebiasis. Disorders of decreased absorption include osmotic, anatomic derangement, and motility disorders. Osmotic diarrhea can occur as a result of digestive abnormalities such as lactose intolerance. Anatomic derangement results in a decreased absorption surface caused by such

procedures as subtotal colectomy and gastrocolic fistula. Motility disorders result from decreased contact time resulting from such diseases as hyperthyroidism and irritable bowel syndrome. Secretory diarrhea is characterized by the hypersecretion of fluid and electrolytes from the cells of the intestinal wall. In classical form, the hypersecretion is due to changes which are independent of the permeability, absorptive capacity and exogenously generated osmotic gradients within the intestine. As discussed above, however, all forms of diarrhea may actually manifest a secretory component.

The methods and products of the invention are particularly useful in treating diarrhea which is secretory. However, the methods and products of the invention may also be used in combination with other treatment methods which are known in the art to treat diarrhea caused by decreased absorption or inflammation. The compounds of the invention are involved in regulating Cl^- secretion and can function alone or when used in combination with other treatment methods to decrease net fluid secretion even when this is due primarily to abnormalities in absorption or inflammation.

The methods and products of the invention are useful in preventing diarrhea and scours in subjects at risk of developing these disorders. Subjects at risk of developing diarrhea and scours are those subjects which have a high likelihood of exposure to the bacterial and viral microorganisms which cause these symptoms. For example, approximately 1/3 of travelers to developing countries will develop diarrhea; infection with rotavirus is one of the leading causes of death in infants in developing countries; subjects with HIV have a greater than 50% chance of developing diarrhea, and many newborn calves and pigs develop scours. Subjects with inflammatory bowel disease develop recurrent diarrhea.

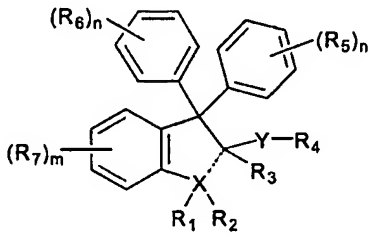
The methods and products of the invention are also useful in treating subjects who already exhibit the symptoms of diarrhea and scours. Once a subject has been exposed to a microorganism causing the symptoms, the subject may be treated with the methods and products of the present invention in order to reduce the symptoms. The symptoms of diarrhea include bowel irregularity, fecal fluid rich in sodium or potassium, fluid feces, dehydration, fever, loss of body weight, headache, anorexia, vomiting, malaise and myalgia. The symptoms of scours include a loss of body weight or failure to grow, dehydration, malodorous feces, fluid feces, feces containing pieces of partially digested milk or semisolid material, and feces of a yellow-white or gray color.

The Compounds

The compounds which are potent, selective and safe inhibitors of Ca^{2+}-activated potassium channel (Gardos channel) of erythrocytes, particularly sickle erythrocytes, mammalian cell proliferation, particularly mitogen-induced cell proliferation, and/or secretagogue-stimulated transepithelial electrogenic chloride secretion in intestinal cells according to the invention, are generally substituted 3,3-diphenyl indanone, indane and *(3-H)* indole compounds, as well as analogues of these classes of compounds wherein the atoms at ring positions 1 and 2 are connected via a double bond.

In one illustrative embodiment, the compounds capable of inhibiting the Gardos channel, mammalian cell proliferation and/or chloride secretion in intestinal cells according to the invention are compounds having the structural formula:

(I)



or pharmaceutically acceptable salts or hydrates thereof, wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', -NR'_2, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -$C(O)NR'_2$ or -$C(S)NR'_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen,

-R', -OR', -SR', -NR'_2, -ONR'_2, -SNR'_2, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -$C(O)NR'_2$, -$C(S)NR'_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -$CH(CN)_2$, -$CH[C(O)R']_2$, -$CH[C(S)R']_2$, -$CH[C(O)OR']_2$, -$CH[C(S)OR']_2$, -$CH[C(O)SR']_2$ and -$CH[C(S)SR']_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -$C(O)NR'_2$, -$C(S)NR'_2$, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -$C(O)NR'_2$, -$C(S)NR'_2$ and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and

--- designates a single or double bond.

In the compounds of structural formula (I), the bond between the atoms at ring positions 1 and 2 (designated ---) can be either a single or double bond. It will be recognized by those of skill in the art that when the bond is a double bond, certain of the substituents must be absent. It will also be recognized that the identity of X also influences the presence or absence of certain substituents. Thus, it is to be understood that when X is N and --- is a double bond, R_1, R_2 and R_3 are absent; when X is C and --- is a double bond, R_2 and R_3 are absent. When X is N and --- is a single bond, one of R_1 and R_2 is present and the other is absent and R_3 is present; when X is C and --- is a single bond, R_1, R_2 and R_3 are each present.

In a preferred embodiment of the invention, the chalcogens in the compounds of formula (I) are each oxygen.

In another preferred embodiment of the invention, the compounds are those of structural formula (I) wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, =O, =N-OR, -O-C(O)R, or when taken together with R_2 is a 3-8 membered oxirane or a substituted 3-8 membered oxirane;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', $-NR'_2$, -CN, $-NO_2$, (C_3-C_8) cycloalkyl, 3-8 membered oxiranyl, 5-8 membered dioxycycloalkyl, -C(O)R', -C(O)OR' or $-C(O)NR'_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', $-NR'_2$, $-ONR'_2$, $-NO_2$, -CN, -C(O)R', -C(O)OR', $-C(O)NR'_2$, -C(O)NR'(OR'), $-CH(CN)_2$, $-CH[C(O)R']_2$ and $-CH[C(O)OR']_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl;

the oxirane substituents are each independently selected from the group consisting of -CN, $-NO_2$, $-NR'_2$, -OR', $-C(O)NR'_2$, -C(O)OR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(O)OR', $-C(O)NR'_2$ and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and/or

--- designates a single or double bond.

In another preferred embodiment, the compounds are those of structural formula (I) wherein:

m is 0 or 1;

each n is independently 0 or 1;

X is C or N;

Y is absent, (C_1-C_3) alkyl, (C_1-C_3) alkenyl or (C_1-C_3) alkynyl;

R_1 is absent -H, -OR, =O, $-NR_2$, =N-OR, -O-C(O)R, or when taken together with R_2 is 3-5 membered oxirane or 3-5 membered substituted oxirane;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR, $-NR_2$, -CN, -C(O)OR, $-C(O)NR_2$ or 5-6 membered dioxoycycloalkyl;

each R_5, R_6 and R_7 is independently selected from the group consisting of -R', -F, -Cl or -Br;

each R is independently selected from the group consisting of -H, (C_1-C_3) alkyl, (C_1-C_3) alkenyl, (C_1-C_3) alkynyl, (C_5-C_{10}) aryl, substituted (C_5-C_{10}) aryl, (C_6-C_{13}) alkaryl, substituted C_6-C_{13}) alkaryl;

the oxirane substituent is -CN, -NO_2, -NR'_2, -OR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of -F, -Cl, -Br, -CN, -NO_2, -NR'_2, -C(O)R', -C(O)OR' and trihalomethyl;

R' is -H, (C_1-C_3) alkyl, (C_1-C_3) alkenyl or (C_1-C_3) alkynyl; and/or

--- is a single or double bond.

In still another preferred embodiment, the compounds are those of structural formula (I) wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', -NR'_2, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -$C(O)NR'_2$ or -$C(S)NR'_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'_2, -ONR'_2, -SNR'_2, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -$C(O)NR'_2$, -$C(S)NR'_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -$CH(CN)_2$, -$CH[C(O)R']_2$, -$CH[C(S)R']_2$, -$CH[C(O)OR']_2$, -$CH[C(S)OR']_2$, -$CH[C(O)SR']_2$ and -$CH[C(S)SR']_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -$C(O)NR'_2$, -$C(S)NR'_2$, -C(O)OR', -C(S)OR', -C(O)SR',

-C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', $-C(O)NR'_2$, $-C(S)NR'_2$ and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl;

--- designates a single or double bond; and

wherein when X is C and R_1 is =O, =S or -OR', at least one of R_5, R_6 or R_7 is other than -R', preferably other than -H, or Y is present or R_4 is other than -H; and when X is N, --- is a double bond and R_1, R_2, R_3 and Y are absent, R_4 is other than $-NR'_2$, preferably other than $-NH_2$.

In still another preferred embodiment, the compounds are those of structural formula (I) wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', $-NR'_2$, -CN, $-NO_2$, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', $-C(O)NR'_2$ or $-C(S)NR'_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', $-NR'_2$, $-ONR'_2$, $-SNR'_2$, $-NO_2$, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', $-C(O)NR'_2$, $-C(S)NR'_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), $-CH(CN)_2$, $-CH[C(O)R']_2$, $-CH[C(S)R']_2$, $-CH[C(O)OR']_2$, $-CH[C(S)OR']_2$, $-CH[C(O)SR']_2$ and $-CH[C(S)SR']_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and

substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -C(O)NR'_2, -C(S)NR'_2, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'_2, -C(S)NR'_2 and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl;

--- designates a single or double bond; and

wherein when R_1 is =O or -OH, at least one of R_5, R_6 or R_7 is other than -R', preferably other than -H, or Y is present or R_4 is other than -H.

As used herein, the term "alkyl" refers to a saturated branched, straight chain or cyclic hydrocarbon radical. Typical alkyl groups include methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, *t*-butyl, cyclobutyl, pentyl, isopentyl, cyclopentyl, hexyl, cyclohexyl and the like.

As used herein, the term "heterocycloalkyl" refers to a saturated cyclic hydrocarbon radical wherein one or more of the carbon atoms is replaced with another atom such as Si, Ge, N, O, S or P. Typical heterocycloalkyl groups include, but are not limited to, morpholino, thiolino, piperidyl, pyrrolidinyl, piperazyl, pyrazolidyl, imidazolidinyl, and the like.

As used herein, the term "alkenyl" refers to an unsaturated branched, straight chain or cyclic hydrocarbon radical having at least one carbon-carbon double bond. The radical may be in either the *cis* or *trans* conformation about the double bond(s). Typical alkenyl groups include ethenyl, propenyl, isopropenyl, cyclopropenyl, butenyl, isobutenyl, cyclobutenyl, *tert*-butenyl, pentenyl, hexenyl and the like.

As used herein, the term "alkynyl" refers to an unsaturated branched, straight chain or cyclic hydrocarbon radical having at least one carbon-carbon triple bond. Typical alkynyl groups include ethynyl, propynyl, butynyl, isobutynyl, pentynyl, hexynyl and the like.

As used herein, the term "alkoxy:" refers to an -OR radical, where R is alkyl, alkenyl or alkynyl, as defined above.

As used herein, the term "aryl" refers to an unsaturated cyclic hydrocarbon radical having a conjugated π electron system. Typical aryl groups include, but are not limited to,

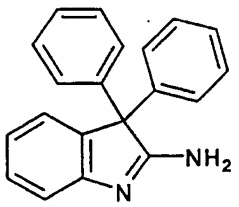
penta-2,4-diene, phenyl, naphthyl, anthracyl, azulenyl, indacenyl, and the like.

As used herein, the term "heteroaryl" refers to an aryl group wherein one or more of the ring carbon atoms is replaced with another atom such as N, O or S. Typical heteroaryl groups include, but are not limited to,furanyl, imidazole, pyridinyl, thiophenyl, indolyl, imidazolyl, quinolyl, thienyl, indolyl, pyrrolyl, pyranyl, pyridyl, pyrimidyl, pyrazyl, pyridazyl, and the like.

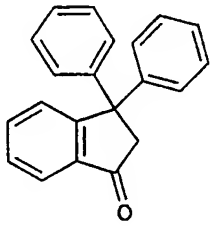
As used herein, the term "heteroarylium" refers to a heteroaryl group wherein one or more hydrogens has been added to any position of the neutral parent ring. Typical heteroarylium groups include, but are not limited to, pyridinium, pyrazinium, pyrimidinium, pyridazinium, 1,3,5-triazinium, and the like.

As used herein, the term "*in situ*" refers to and includes the terms "*in vivo*," "*ex vivo*," and "*in vitro*" as these terms are commonly recognized and understood by persons ordinarily skilled in the art. Moreover, the phrase "*in situ*" is employed herein in its broadest connotative and denotative contexts to identify an entity, cell or tissue as found or in place, without regard to its source or origin, its condition or status or its duration or longevity at that location or position.

In still another preferred embodiment, the compounds of structural formula (I) are selected from the group of compounds set forth below:



(1)



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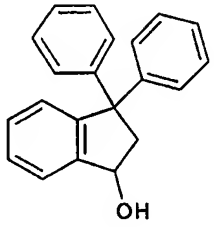

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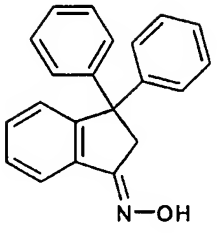

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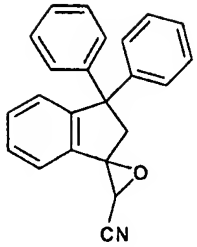

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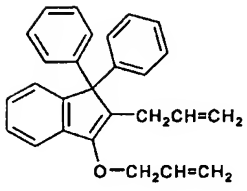

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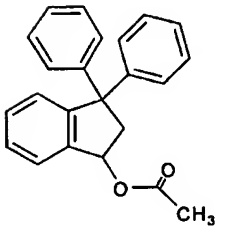

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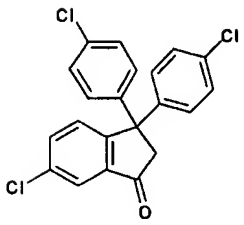

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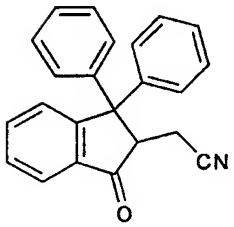

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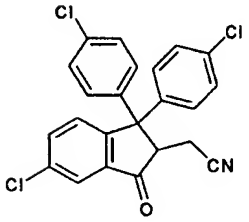

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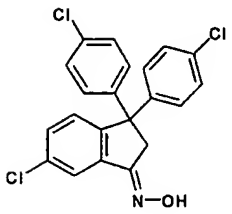

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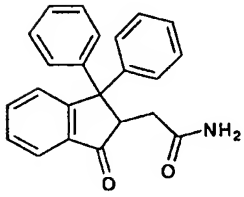

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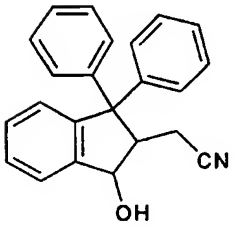

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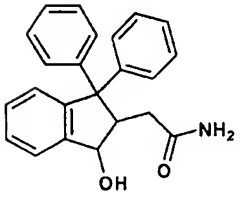

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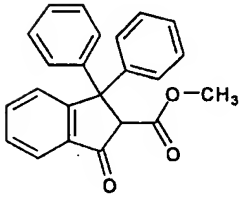

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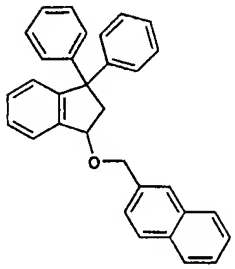

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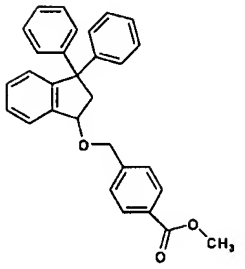

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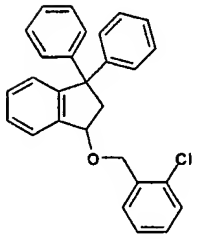

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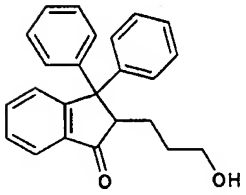

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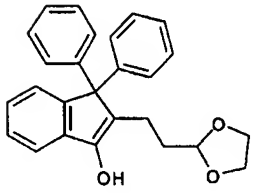


(20)

The compounds will be referred to herein by way of compound numbers as presented above.

In still another preferred embodiment, the compounds of structural formula (I) are selected from the group consisting of Compounds **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **19** and **20**.

The chemical formulae referred to herein may exhibit the phenomena of tautomerism or conformational isomerism. As the formulae drawings within this specification can represent only one of the possible tautomeric or conformational isomeric forms, it should be understood that the invention encompasses any tautomeric or conformational isomeric forms which exhibit biological or pharmacological activity as described herein.

The compounds of the invention may be in the form of free acids, free bases or pharmaceutically effective salts thereof. Such salts can be readily prepared by treating a compound with an appropriate acid. Such acids include, by way of example and not limitation, inorganic acids such as hydrohalic acids (hydrochloric, hydrobromic, etc.), sulfuric acid, nitric acid, phosphoric acid, etc.; and organic acids such as acetic acid, propanoic acid, 2-hydroxyacetic acid, 2-hydroxypropanoic acid, 2-oxopropanoic acid, propandioic acid, butandioic acid, etc. Conversely, the salt can be converted into the free base form by

treatment with alkali.

In addition to the above-described compounds and their pharmaceutically acceptable salts, the invention may employ, where applicable, solvated as well as unsolvated forms of the compounds (*e.g.* hydrated forms).

The compounds described herein may be prepared by any processes known to be applicable to the preparation of chemical compounds. Suitable processes are well known in the art. Preferred processes are illustrated by the representative examples. Necessary starting materials may be obtained commercially or by standard procedures of organic chemistry. Synthetic protocols for these compounds are described in US application serial no. 08/975,595, filed November 20, 1997, entitled "Methods for Treatment or Prevention of Sickle Cell Disease with Substituted Diphenyl Indane and Indole Compounds and Analogs Thereof", the disclosure of which is incorporated herein by reference.

An individual compound's relevant activity and potency as an agent to affect sickle cell dehydration or deformation, mammalian cell proliferation and/or secretagogue-stimulated transepithelial electrogenic chloride secretion in intestinal cells may be determined using standard techniques. Preferentially, a compound is subject to a series of screens to determine its pharmacological activity.

In most cases, the active compounds of the invention exhibit three pharmacological activities: inhibition of the Gardos channel of erythrocytes, inhibition of secretagogue-stimulated transepithelial electrogenic chloride secretion in intestinal cells and inhibition of mammalian cell proliferation. However, in some cases, the compounds of the invention may exhibit only one of these pharmacological activities. Any compound encompassed by formula (I) which exhibits at least one of these pharmacological activities is considered to be within the scope of the present invention.

In general, the active compounds of some embodiments of the invention are those which induce at least about 25% inhibition of the Gardos channel of erythrocytes (measured at about 10 μM), at least about 25% inhibition of secretagogue-stimulated transepithelial electrogenic chloride secretion in intestinal cells (measured at about 10 μM) and/or about 25% inhibition of mammalian cell proliferation (measured at about 10 μM), as measured using *in vitro* assays that are commonly known in the art (*see, e.g.*, Brugnara *et al.*, 1993, J. Biol. Chem. 268(12):8760-8768; Benzaquen *et al.*, 1995, Nature Medicine 1:534-540). Alternatively, or in addition, the active compounds of the invention generally will have an

IC_{50} (concentration of compound that yields 50% inhibition) for inhibition of the Gardos channel of erythrocytes of less than about 10 μM, an IC_{50} for secretagogue-stimulated transepithelial electrogenic chloride secretion in intestinal cells of less than about 10 μM, and/or an IC_{50} for inhibition of cell proliferation of less than about 10 μM, as measured using *in vitro* assays that are commonly known in the art (*see, e.g.*, Brugnara *et al.*, 1993, J. Biol. Chem. 268(12):8760-8768; Benzaquen *et al.*, 1995, Nature Medicine 1:534-540) and the Examples section below. Other assays for assessing the activity and/or potency of an agent with respect to the uses of the invention are described below with respect to an effective amount of the compounds.

Representative active compounds according to the invention are Compounds **1** through **20**, as illustrated above.

In certain embodiments of the invention, compounds which exhibit only one pharmacological activity, or a higher degree of one activity, may be preferred. Thus, when the compound is to be used in methods to treat or prevent sickle cell disease, or in methods to reduce sickle cell dehydration and/or delay the occurrence of erythrocyte sickling or deformation *in situ*, it is preferred that the compound exhibit at least about 75% Gardos channel inhibition (measured at about 10 μM).

Exemplary preferred compounds for use in methods related to Gardos channel inhibition and sickle cell disease include Compounds **1, 2, 3, 4, 7, 9, 12, 13** and **14**.

When the compound is to be used in methods to treat or prevent diarrhea and/or scours, it is preferred that the compound exhibit at least about 75% inhibition of Cl^- secretion from intestinal cells (measured at about 10 μM) and/or have an IC_{50} of inhibition of Cl^- secretion from intestinal cells of less than about 1 μM, with at least about 90% inhibition and/or an IC_{50} of less than about 0.1 μM being particularly preferred.

When the compound is to be used in methods to treat or prevent disorders characterized by abnormal cell proliferation or in methods to inhibit cell proliferation *in situ*, it is preferable that the compound exhibit at least about 75% inhibition of mitogen-induced cell proliferation (measured at about 10 μM) and/or have an IC_{50} of cell proliferation of less than about 3.5 μM, with at least about 90% inhibition and/or an IC_{50} of less than about 1 μM being particularly preferred. Even more preferred compounds meet both the % inhibition and IC_{50} criteria.

Exemplary preferred compounds for use in methods inhibiting mammalian cell

proliferation or for the treatment or prevention of diseases characterized by abnormal cell proliferation include compound numbers **1, 2, 3, 4, 6, 7, 8, 10, 11, 15, 16, 17, 19** and **20**.

Formulation and Routes of Administration

The compounds described herein, or pharmaceutically acceptable addition salts or hydrates thereof, can be delivered to a patient using a wide variety of routes or modes of administration. Suitable routes of administration include, but are not limited to, inhalation, transdermal, oral, rectal, transmucosal, intestinal and parenteral administration, including intramuscular, subcutaneous and intravenous injections.

The compounds described herein, or pharmaceutically acceptable salts and/or hydrates thereof, may be administered singly, in combination with other compounds of the invention, and/or in cocktails combined with other therapeutic agents. Of course, the choice of therapeutic agents that can be co-administered with the compounds of the invention will depend, in part, on the condition being treated.

A subject as used herein, means humans, primates, horses, cows, sheep, pigs, goats, cats and dogs.

For example, when administered to subjects undergoing cancer treatment, the compounds may be administered in cocktails containing other anti-cancer agents and/or supplementary potentiating agents. The compounds may also be administered in cocktails containing agents that treat the side-effects of radiation therapy, such as anti-emetics, radiation protectants, etc.

Anti-cancer drugs that can be co-administered with the compounds of the invention include, e.g., Aminoglutethimide; Asparaginase; Bleomycin; Busulfan; Carboplatin; Carmustine (BCNU); Chlorambucil; Cisplatin (cis-DDP); Cyclophosphamide; Cytarabine HCl; Dacarbazine; Dactinomycin; Daunorubicin HCl; Doxorubicin HCl; Estramustine phosphate sodium; Etoposide (VP-16); Floxuridine; Fluorouracil (5-FU); Flutamide; Hydroxyurea (hydroxycarbamide); Ifosfamide; Interferon Alfa-2a, Alfa 2b, Lueprolide acetate (LHRH-releasing factor analogue); Lomustine (CCNU); Mechlorethamine HCl (nitrogen mustard); Melphalan; Mercaptopurine; Mesna; Methotrexate (MTX); Mitomycin; Mitotane (o.p'-DDD); Mitoxantrone HCl; Octreotide; Plicamycin; Procarbazine HCl; Streptozocin; Tamoxifen citrate; Thioguanine; Thiotepa; Vinblastine sulfate; Vincristine sulfate; Amsacrine (m-AMSA); Azacitidine; Hexamethylmelamine (HMM); Interleukin 2; Mitoguazone (methyl-

GAG; methyl glyoxal bis-guanylhydrazone; MGBG); Pentostatin; Semustine (methyl-CCNU); Teniposide (VM-26); paclitaxel and other taxanes; and Vindesine sulfate.

Supplementary potentiating agents that can be co-administered with the compounds of the invention include, e.g., Tricyclic anti-depressant drugs (e.g., imipramine, desipramine, amitriptyline, clomipramine, trimipramine, doxepin, nortriptyline, protriptyline, amoxapine and maprotiline); non-tricyclic and anti-depressant drugs (e.g., sertraline, trazodone and citalopram); Ca^{++} antagonists (e.g., verapamil, nifedipine, nitrendipine and caroverine); Amphotericin (e.g., Tween 80 and perhexiline maleate); Triparanol analogues (e.g., tamoxifen); antiarrhythmic drugs (e.g., quinidine); antihypertensive drugs (e.g., reserpine); Thiol depleters (e.g., buthionine and sulfoximine); and calcium leucovorin.

The active compound(s) may be administered *per se* or in the form of a pharmaceutical composition wherein the active compound(s) is in admixture with one or more pharmaceutically acceptable carriers, excipients or diluents. Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium

carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration,the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or

dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or transcutaneous delivery (for example subcutaneously or intramuscularly), intramuscular injection or a transdermal patch. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

One product of the invention is a veterinary preparation of an aromatic compound of the invention, used alone or combined with an anti-scours agent. An anti-scours agent is a composition which is known to be useful in preventing or inhibiting the symptoms of scours. Known compositions include, for example, colostral extracts, such as those described in U.S. patent no. 4,377,569 and Canadian patent no. 1,175,352 and widely commercially available (e.g. Soluble Colostrum Powder, by VedCo, Inc., St. Joseph MO; Colostrum Bolus II, by RX

Veterinary Products, Kansas City MO, etc.); an immunological preparation of colostrum isolated from milk-producing mammals which may have been immunized against certain diarrheal causing microorganisms, such as those described in U.S. patent no. 4,834,974, Australian patent no. 39340/89, Australian patent no. 52547/90, and German patent no. 1,560,344; microorganism specific immunological preparations, including microorganism specific hybridoma-derived monoclonal antibodies such as those described in Sherman et al., Infection and Immunity, V. 42 (2), P. 653-658 (1983) and a bovine immunoglobulin fraction prepared from bovine plasma or clear bovine serum such as the fraction described in U.S. patent no. 3,984,539; oral rehydration fluids and/or replacement electrolyte compositions which are widely commercially available in the form of dry compositions or liquid solutions prepared for oral or intravenous administration (e.g. Electrolyte H, by Agri-Pet Inc., Aubrey TX; Electrolyte Powder 8x, by Phoenix Pharmaceutical Inc, St. Joseph MO; Electrolyte Solution Rx, by Lextron Inc., Greeley CO, ProLabs LTD, St. Joseph MO, and VetTek Inc., Blue Springs MO; Calf Rehydrate, by Durvet Inc., Blue Springs MO, etc.) and antibiotic compositions which are commercially available (e.g. BIOSOL® Liquid, by The UpJohn Company Animal Health Division, Kalamazoo MI; AMOXI-BOL®, by SmithKline-Beecham Animal Health, Exton PA; 5-WAY CALF SCOUR BOLUS™, by Agri Laboratories LTD, St. Joseph MO; 1-A-DAY CALF SCOUR BOLUS, by A.H.A.; GARACIN® PIG PUMP, by Schering-Plough Animal Health Corporation, Kenilworth NJ, etc.).

In one embodiment, the veterinary preparation is a dry preparation of the aromatic compound of the invention and an antiscours agent. The dry preparation may be administered directly or may be hydrated and/or diluted in a liquid solution prior to administration. In another embodiment, the veterinary preparation is a liquid solution of the compound of the invention and an anti-scours agent.

Another product of the invention is a pharmaceutical preparation of an aromatic compound of the invention and an anti-diarrheal agent. An anti-diarrheal agent includes, for example, an immunoglobulin preparation from bovine colostrum; lomotil; an intravenous or oral rehydration fluid; a dry rehydration composition salt; an electrolyte replacement composition (in dry or liquid form); an oral or intravenous sugar-electrolyte solution or dry composition; an antibiotic such as tetracycline, trimethoprim or sulfamethoxazole; a quinolone drug such as norfloxacin or ciprofloxacin, bismuth subsalicylate, diphenoxylate; and loperamide.

In one embodiment the pharmaceutical preparation is a dry preparation of the aromatic compound of the invention and an anti-diarrheal agent. The dry preparation may be administered directly or may be hydrated and/or diluted in a liquid solution prior to administration. In another embodiment the pharmaceutical preparation is a liquid solution of the aromatic compound of the invention and an anti-diarrheal agent.

The time of administration of the aromatic compounds useful according to the invention varies depending upon the purpose of the administration. When the compounds of the invention are administered in order to prevent the development of diarrhea in subjects traveling to areas with high risk of exposure to infectious agent or subjects otherwise exposed to diarrhea causing agents, the compounds should be administered at about the time that the subject is exposed to the risk or the high risk area. When the compounds are administered to subjects in order to prevent the development of scours, the veterinary preparation should be administered within the first 12 hours after birth, and preferably within the first 4 hours after birth. When the compounds of the invention are used to treat subjects having symptoms of diarrhea or scours, the compounds may be administered at any point while the subject is experiencing symptoms, and preferably as soon as the symptoms develop. Other considerations will be apparent when the compounds are used to treat inflammatory diseases, proliferative diseases, etc.

When administered, the formulations of the invention are applied in pharmaceutically acceptable amounts and in pharmaceutically acceptable compositions. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic ingredients. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulfonic, tartaric, citric, methane sulfonic, formic, malonic, succinic, naphthalene-2-sulfonic, and benzene sulfonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and a salt (1-2% W/V); citric acid and a salt (1-3% W/V); boric acid and a salt (0.5-2.5% W/V); and phosphoric acid and a salt

(0.8-2% W/V).

Suitable preservatives include benzalkonium chloride (0.003-0.03% W/V); chlorobutanol (0.3-0.9% W/V); parabens (0.01-0.25% W/V) and thimerosal (0.004-0.02% W/V).

The active compounds of the present invention may be pharmaceutical compositions having a therapeutically effective amount of an aromatic compound of the general formula provided above in combination with a non-formula (I) active agent, optionally included in a pharmaceutically-acceptable carrier. The active compounds of the present invention also may be veterinary compositions having a therapeutically effective amount of an aromatic compound of the general formula provided above in combination with a non-formula (I) active agent, optionally included in a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid filler, dilutants or encapsulating substances which are suitable for administration to a human or other animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compound of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

A common administration vehicle (e.g., pill, tablet, bolus, powder or solution for dilution, pig pump, implant, injectable solution, etc.) would contain both the compounds useful in this invention and a non-formula (I) active agent. Thus, the present invention provides pharmaceutical or veterinary compositions, for medical or veterinary use, which comprise the active compounds of the invention together with one or more pharmaceutically acceptable carriers thereof and other therapeutic ingredients.

A variety of administration routes are available. The particular mode selected will depend of course, upon the particular drug selected, the severity of the condition being treated and the dosage required for therapeutic efficacy. The methods of this invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, topical, nasal, transdermal or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intramuscular, or infusion. Intravenous and intramuscular routes are not

particularly suited for long term therapy and prophylaxis. They could, however, be preferred in emergency situations. Oral administration will be preferred for prophylactic treatment because of the convenience to the subject as well as the dosing schedule.

The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compounds into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compounds into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active compound, which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using those suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1, 3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Carrier formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. can be found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the active compounds of the invention, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer based systems such as polylactic and polyglycolic acid, polyanhydrides and polycaprolactone; nonpolymer systems that are lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-, di- and triglycerides; hydrogel release systems; silastic systems; peptide based systems; wax coatings, compressed tablets using conventional binders and excipients, partially fused implants and the like. Specific examples include, but are not limited to: (a) erosional systems in which an agent of the

invention is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Use of a long-term sustained release implant may be particularly suitable for treatment of diarrhea in immunodeficient subjects, who need continuous administration of the compositions of the invention. "Long-term" release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well known to those of ordinary skill in the art and include some of the release systems described above.

Effective Dosages

Pharmaceutical compositions suitable for use with the present invention include compositions wherein the active ingredient is contained in a therapeutically effective amount, *i.e.*, in an amount effective to achieve its intended purpose. Of course, the actual amount effective for a particular application will depend, *inter alia*, on the condition being treated. For example, when administered in methods to reduce sickle cell dehydration and/or delay the occurrence of erythrocyte sickling or distortion *in situ*, such compositions will contain an amount of active ingredient effective to achieve this result. When administered in methods to inhibit cell proliferation, such compositions will contain an amount of active ingredient effective to achieve this result. When administered to subjects suffering from disorders characterized by abnormal cell proliferation, such compositions will contain an amount of active ingredient effective to, *inter alia*, prevent the development of or alleviate the existing symptoms of, or prolong the survival of, the subject being treated. For use in the treatment of cancer, a therapeutically effective amount further includes that amount of compound which arrests or regresses the growth of a tumor. Determination of an effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

For any compound described herein the therapeutically effective amount can be initially determined from cell culture assays. Target plasma concentrations will be those concentrations of active compound(s) that are capable of inducing at least about 25%

inhibition of the Gardos channel, at least about 25% inhibition of Cl^- secretion in intestinal cells and/or at least about 25% inhibition of cell proliferation in cell culture assays, depending, of course, on the particular desired application. Target plasma concentrations of active compound(s) that are capable of inducing at least about 50%, 75%, or even 90% or higher inhibition of the Gardos channel, of Cl^- secretion in intestinal cells, and/or of cell proliferation in cell culture assays are preferred. The percentage inhibition of the Gardos channel, of Cl^- secretion in intestinal cells, and/or cell proliferation in the subject can be monitored to assess the appropriateness of the plasma drug concentration achieved, and the dosage can be adjusted upwards or downwards to achieve the desired percentage of inhibition.

Therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a circulating concentration that has been found to be effective in animals. Useful animal models for diseases characterized by abnormal cell proliferation are well-known in the art. A particularly useful animal model for sickle cell disease is the SAD mouse model (Trudel *et al.*, 1991, EMBO J. 11:3157-3165). Useful animal models for diseases characterized by abnormal cell proliferation are well-known in the art. In particular, the following references provide suitable animal models for cancer xenografts (Corbett *et al.*, 1996, J. Exp. Ther. Oncol. 1:95-108; Dykes *et al.*, 1992, Contrib. Oncol. Basel. Karger 42:1-22), restenosis (Carter *et al.*, 1994, J. Am. Coll. Cardiol. 24(5):1398-1405), atherosclerosis (Zhu *et al.*, 1994, Cardiology 85(6):370-377) and neovascularization (Epstein *et al.*, 1987, Cornea 6(4):250-257). The dosage in humans can be adjusted by monitoring Gardos channel inhibition and/or inhibition of cell proliferation and adjusting the dosage upwards or downwards, as described above.

Additional *in vivo* assays are well known in the art. For instance, the following assays are useful for assessing effective amounts of compounds for treating inflammatory diseases associated with cellular proliferation: Airway inflammation and hyperresponsiveness in Ovalbumin-sensitized mice or guinea pigs; NZB/NZW crossed mice develop glomerular disease and lupus-like syndrome; Renal allograft rejection in mice; Trinitrobenzene sulphonic acid induced bowel inflammation in rats; NZB/NZW crossed mice develop glomerular disease and lupus-like syndrome; Experimental allergic encephalomyelitis; Rat adjuvant arthritis assay; HLA transgenic mice immunized with thyroglobulin; and Thiouracil-fed rats.

A therapeutically effective dose can also be determined from human data for compounds which are known to exhibit similar pharmacological activities, such as

Clotrimazole and other antimycotic agents (*see, e.g.*, Brugnara *et al.*, 1995, JPET 273:266-272; Benzaquen *et al.*, 1995, Nature Medicine 1:534-540; Brugnara *et al.*, 1996, J. Clin. Invest. 97(5):1227-1234). The applied dose can be adjusted based on the relative bioavailability and potency of the administered compound as compared with Clotrimazole.

Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods as are well-known in the art is well within the capabilities of the ordinarily skilled artisan.

Of course, in the case of local administration, the systemic circulating concentration of administered compound will not be of particular importance. In such instances, the compound is administered so as to achieve a concentration at the local area effective to achieve the intended result.

For use in the prophylaxis and/or treatment of sickle cell disease, including both chronic sickle cell episodes and acute sickle cell crisis, a circulating concentration of administered compound of about 0.001 μM to 20 μM is considered to be effective, with about 0.1 μM to 5 μM being preferred.

Subject doses for oral administration of the compounds described herein, which is the preferred mode of administration for prophylaxis and for treatment of chronic sickle cell episodes, typically range from about 80 mg/day to 16,000 mg/day, more typically from about 800 mg/day to 8000 mg/day, and most typically from about 800 mg/day to 4000 mg/day. Stated in terms of subject body weight, typical dosages range from about 1 to 200 mg/kg/day, more typically from about 10 to 100 mg/kg/day, and most typically from about 10 to 50 mg/kg/day. Stated in terms of subject body surface areas, typical dosages range from about 40 to 8000 mg/m^2/day, more typically from about 400 to 4000 mg/m^2/day, and most typically from about 400 to 2000 mg/m^2/day.

For use in the treatment of disorders characterized by abnormal cell proliferation, including cancer, arteriosclerosis and angiogenic conditions such as restenosis, a circulating concentration of administered compound of about 0.001 μM to 20 μM is considered to be effective, with about 0.1 μM to 5 μM being preferred.

Subject doses for oral administration of the compounds described herein for the treatment or prevention of cell proliferative disorders typically range from about 80 mg/day to 16,000 mg/day, more typically from about 800 mg/day to 8000 mg/day, and most typically from about 800 mg/day to 4000 mg/day. Stated in terms of subject body weight, typical

dosages range from about 1 to 200 mg/kg/day, more typically from about 10 to 100 mg/kg/day, and most typically from about 10 to 50 mg/kg/day. Stated in terms of subject body surface areas, typical dosages range from about 40 to 8000 mg/m^2/day, more typically from about 400 to 4000 mg/m^2/day, and most typically from about 400 to 2000 mg/m^2/day.

For other modes of administration, dosage amount and interval can be adjusted individually to provide plasma levels of the administered compound effective for the particular clinical indication being treated. For example, if acute sickle crises are the most dominant clinical manifestation, a compound according to the invention can be administered in relatively high concentrations multiple times per day. Alternatively, if the subject exhibits only periodic sickle cell crises on an infrequent or periodic or irregular basis, it may be more desirable to administer a compound of the invention at minimal effective concentrations and to use a less frequent regimen of administration. This will provide a therapeutic regimen that is commensurate with the severity of the sickle cell disease state.

For use in the treatment of tumorigenic cancers, the compounds can be administered before, during or after surgical removal of the tumor. For example, the compounds can be administered to the tumor via injection into the tumor mass prior to surgery in a single or several doses. The tumor, or as much as possible of the tumor, may then be removed surgically. Further dosages of the drug at the tumor site can be applied post removal. Alternatively, surgical removal of as much as possible of the tumor can precede administration of the compounds at the tumor site.

Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, subject body weight, severity of adverse side-effects and preferred mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the clinical symptoms demonstrated by the particular subject. Of course, many factors are important in determining a therapeutic regimen suitable for a particular indication or subject. Severe indications such as cancer may warrant administration of higher dosages as compared with less severe indications such as sickle cell disease.

The formulations of the invention are also administered in effective amounts when treating diarrhea or scours. An effective amount is one sufficient to inhibit or prevent diarrhea or scours and is thus sufficient to inhibit the Cl^- secretion of intestinal epithelial cells. An

amount which is sufficient to inhibit the Cl^- secretion of intestinal epithelial cells thereby effectively decreases the secretory response, thereby resulting in a decrease in diarrhea or scours and/or the symptoms thereof. Effective amounts will depend, of course, on the particular condition being treated; the severity of the condition; individual subject parameters including age, physical condition, size and weight; concurrent treatment; frequency of treatment; and the mode of administration. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

An effective amount for an individual compound may be assesed using any method known in the art which reliably determines the amount of Cl^- secretion from intestinal cells. A compound may be subject to a series of standard assays or screens to determine its pharmacological activity and effective amounts.

In general, the active compounds of the invention are those which induce at least about 25% inhibition of the Cl^- secretion, as measured using *in vitro* assays that are commonly known in the art (*see, e.g.*, Example 4). Alternatively, or in addition, the active compounds of the invention generally will have an IC_{50} (concentration of compound that yields 50% inhibition) for inhibition of the Cl^- secretion of less than about 10 μM as measured using *in vitro* assays.

It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment, particularly if acute diarrhea or scours are the dominant clinical manifestation. Dosage may be adjusted appropriately to achieve desired drug plasma levels. Generally, daily oral doses of active compounds will be from about 0.01 milligrams/kg per day to 1000 milligrams/kg per day. It is expected that oral doses in the range of 50 to 500 milligrams/kg, in one or several administrations per day, will yield the desired results. In the event that the response in a subject is insufficient at such doses, even higher doses (or effective higher doses by a different, more localized delivery route) may be employed to the extent that subject tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds.

Toxicity

The ratio between toxicity and therapeutic effect for a particular compound is its therapeutic index and can be expressed as the ratio between LD_{50} (the amount of compound lethal in 50% of the population) and ED_{50} (the amount of compound effective in 50% of the population). Compounds which exhibit high therapeutic indices are preferred. Therapeutic

index data obtained from cell culture assays and/or animal studies can be used in formulating a range of dosages for use in humans. The dosage of such compounds preferably lies within a range of plasma concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the subject's condition. (See *e.g.* Fingl *et al.*, 1975, *In:* The Pharmacological Basis of Therapeutics, Ch. 1 p1).

The invention having been described, the following examples are intended to illustrate, not limit, the invention. Some of the following examples depict tests that are employed to determine the effects on Cl^- secretion. Clotrimazole, which is outside of the scope of the present claims is used to exemplify how the compounds of the present invention are tested for certain conditions. The compounds of the present invention are structurally distinct from the structure of clotrimazole. Nevertheless, the compounds of the present invention act on chloride secretion in the same manner as clotrimazole and, therefore, are useful in the methods and products of the present invention.

Examples

Example 1. Compound Syntheses

This Example demonstrates general methods for synthesizing the compounds of the invention, as well as preferred methods of synthesizing certain exemplary compounds of the invention. In all of the reaction schemes described herein, suitable starting materials are either commercially available or readily obtainable using standard techniques of organic synthesis. Where necessary, suitable groups and schemes for protecting the various funtionalities are well-known in the art, and can be found, for example, in Kocienski, Protecting Groups, Georg Thieme Verlag, New York, 1994 and Greene & Wuts, Protective Groups in Organic Chemistry, John Wiley & Sons, New York, 1991.

In FIGS. 1 and 2, the various substituents are defined as for structure (I), *supra.*

1. Synthesis of Substituted 3,3-Diphenyl Indanones

Referring to FIG. 1, substituted 3,3-diphenyl indanone compounds are synthesized as follows: substituted triphenylpropionic acid **100** (0.25-0.50 M in sulfuric acid) is stirred at room temperature for 1 hour and then poured into an equal volume of cold water. The aqueous mixture is extracted with an equal volume of ethyl acetate and the organics dried over sodium sulfate. Evaporation gives the desired substituted 3,3-diphenyl indanone compound

102 in about 60-75% yield.

2. Synthesis of Substituted 1-Hydroxy-3,3-Diphenyl Indane Compounds

Referring to FIG. 1, substituted 1-hydroxy-3,3-diphenyl indane compounds are synthesized as follows: a solution of substituted 3,3-diphenylindanone **102** (0.25 M in tetrahydrofuran) is added dropwise to 0.25 volume of a 1.0 M solution of lithium aluminum hydride in tetrahydrofuran at 0-5 °C. The mixture is warmed to reflux and refluxed for 2.5 h, cooled to 0-5 °C and an equal volume of 1 M HCl added slowly. The mixture is then extracted three times with an equal volume of ethyl acetate. The combined organic extracts are washed with a saturated aqueous solution of sodium bicarbonate and dried over sodium sulfate. Evaporation gives the desired substituted 1-hydroxy-3,3-diphenyl indane compound **104** in about 45-90 % yield.

3. Synthesis of Substituted 1-N-Oxime-3,3-Diphenyl Indanes

Referring to FIG. 1, substituted 1-N-oxime-3,3-diphenyl indane compounds are synthesized as follows: substituted 3,3-diphenylindanone **102** (1 equivalent) is combined with 5 equivalents of hydroxylamine hydrochloride and 10 equivalents of sodium acetate and dissolved in methanol. The solution is stirred at room temperature for 16 h and then an equal volume of water is added. The mixture is extracted three times with an equal volume of ethyl acetate and the combined organic extracts are dried over sodium sulfate. Evaporation gives the desired substituted 1-N-oxime-3,3-diphenyl indane compound **106** (as a mixture of cis and trans isomers) in about 90-98 % yield.

4. Synthesis of Substituted 2-Alkyl-3,3-Diphenyl Indanones

Referring to FIG. 1, substituted 2-alkyl-3,3-diphenyl indanone compounds are synthesized as follows: substituted 3,3-diphenyl indanone **102** (1 equivalent) is dissolved in tetrahydrofuran (0.4 - 1.0 M) and 1.2 equivalents of potassium hydride is added. The mixture is stirred at room temperature until the gas evolution subsides and then the bromoalkane (1.2 equivalents) is added. The mixture is stirred at room temperature and monitored by TLC. The reaction is quenched with water and the mixture extracted with ethyl acetate. The desired substituted 2-alkyl-3,3-diphenyl indanone compound **108** is isolated by silica gel chromatography in about 50-75% yield.

5. Synthesis of Substituted 1-Alkoxy-3,3-Diphenyl Indanes

Referring to FIG. 1, substituted 1-alkoxy-3,3-diphenyl indane compounds are synthesized as follows: substituted 1-hydroxy-3,3-diphenylindanone **104** (1 equivalent) is combined with 2 equivalents of sodium hydride in N,N-dimethylformamide and stirred at room temperature until the gas evolution subsides. The haloalkane (2 equivalents) is added and stirred at room temperature for 16-20 hours. An equal volume of water is added and the mixture extracted four times with twice the volume of ethyl acetate. The combined organic extracts are dried over sodium sulfate and the solvent removed *in vacuo*. The desired substituted 1-alkoxy-3,3-diphenyl indane compound **110** is isolated by vacuum distillation.

1.6 Synthesis of Substituted 3,3-Diphenyl-3*H*-Indoles

Referring to FIG. 2, substituted 3,3-diphenyl-3*H*-indole compounds are synthesized as follows: substituted phenyl hydrazine **120** is combined with an equimolar amount of substituted 1,1-diphenyl-2-ketone **122** in phosphoric acid. This mixture is stirred at 100-120 °C until the reaction is complete as determined by TLC. The reaction is cooled to 60-70 °C and diluted with twice the volume of water while stirring. After cooling to room temperature, the mixture is filtered, washed with water, and the crude solid substituted 3,3-diphenyl indole compound **124** is purified by column chromatography or crystallization.

1.7 Synthesis of Substituted 3,3-Diphenyl-3*H*-Indolines

Referring to FIG. 2, substituted 3,3-diphenyl-3*H*-indoline compounds are synthesized as follows: substituted 3,3-diphenyl indole compound **124** is reduced with sodium borohydride or sodium cyanoborohydride in a suitable solvent to yield the substituted 3,3-diphenyl-3*H*-indoline compound **126**.

1.8 Synthesis of Substituted N-Substituted-3,3-Diphenyl Indolines

Referring to FIG. 2, substituted N-substituted-3,3-diphenyl indoline compounds are synthesized as follows: substituted 3,3-diphenyl indoline **126** (1 equivalent) is combined with an alkyl halide (1 equivalent) and potassium carbonate (3-4 equivalents) in acetonitrile. The mixture is stirred at reflux until the reaction is complete as determined by TLC. Water and ethyl acetate are added and the mixture is extracted with ethyl acetate. Evaporation of the combined ethyl acetate extracts gives the crude substituted N-substituted-3,3-diphenyl

indoline compound 128, which is purified by column chromatography.

1.9 Synthesis of 3,3-Diphenylindanone (Compound 2)

3,3-Diphenylindanone (Compound **2**) was synthesized as follows: Triphenylpropionic acid (12 g, 0.04 mol) was stirred in 50 ml concentrated sulfuric acid for 1 hour. The reaction mixture was cooled in an ice bath and diluted with 50 ml water. This mixture was extracted three times with ethyl acetate. The ethyl acetate extracts were combined, dried over sodium sulfate and the solvent removed *in vacuo* to yield 9.0 g (78% yield) of 3,3-Diphenylindanone (Compound **2**) as a white solid having a melting point of 119-123°C.

1.10 Synthesis of 1-Hydroxy-3,3-Diphenylindane (Compound 3)

1-Hydroxy-3,3-Diphenylindane (Compound **3**) was synthesized as follows: A solution of 2 g (0.007 mol) 3,3-diphenylindanone (Compound **2**) in 20 ml of tetrahydrofuran was added dropwise to a solution of 0.34 g (0.009 mol) $LiAlH_4$ in 10 ml tetrahydrofuran at 0-5°C. The mixture was warmed to reflux and refluxed for 3 hr., cooled to 0-5°C and 30 ml of 1 M HCl added slowly. The mixture was then extracted three times with 60 ml ethyl acetate. The ethyl acetate extracts were combined, washed with a saturated aqueous solution of sodium bicarbonate and dried over sodium sulfate. Evaporation of the solvent gave 0.9 g (45% yield) of 1-Hydroxy-3,3-Diphenylindane (Compound **3**) as white crystals with a melting point of 133-135°C.

1.11 Synthesis of 1-N-Oxime-3,3-Diphenylindane (Compound 4)

1-N-Oxime-3,3-Diphenylindane (Compound **4**) was synthesized as follows: 3,3-Diphenylindanone (Compound **2**) (2.0 g, 0.007 mol) was combined with 2.4 g (0.035 mol) of hydroxylamine hydrochloride and 5.8 g (0.07 mol) of sodium acetate and dissolved in 30 ml of methanol. The solution was stirred at room temperture for 16 hr and then 100 ml of water was added. The mixture was extracted with 100 ml ethyl acetate and the organic layer dried over sodium sulfate. Evaporation of the solvent gave 1.9 g (90% yield) of 1-N-Oxime-3,3-Diphenylindane (Compound **4**) as a white solid having a melting point of 138-141°C.

1.12 Synthesis of Spiro[3,3-diphenyl-2,3-dihydro(1H)indene-1,3'-2'-cyanooxirane] (Compound 5) and 2-Cyanomethyl-3,3-diphenylindanone (Compound 9)

Spiro[3,3-diphenyl-2,3-dihydro(1H)indene-1,3'-2'-cyanooxirane] (Compound **5**) and 2-cyanomethyl-3,3-diphenylindanone (Compound **9**) were synthesized as follows: 3,3-diphenylindanone (Compound **2**), 5.0 g (0.0176 mole) and 2.62 g (0.0229 mole) of potassium hydride were stirred at room temperature in 40 mL of tetrahydrofuran. After the gas evolution subsided (approx. 45 min), 1.5 mL (0.0215 mole) of bromoacetonitrile was added. The dark red mixture was stirred for 1 hour and then 50 mL of water was added. The mixture was extracted three times with 75 mL of ethyl acetate. The combined organic extracts were concentrated *in vacuo*, loaded onto a silica gel column and eluted with 10% ethyl acetate in hexane. Three fractions were collected. After evaporation of the solvent, the first fraction yielded unreacted starting material (3.5 g). The second fraction yielded 0.49 g (9% yield) of spiro[3,3-diphenyl-2,3-dihydro(1H)indene-1,3'-2'-cyanooxirane] (Compound **5**) as a white solid. The third fraction yielded 1.05 g (18% yield) of 2-cyanomethyl-3,3-diphenylindanone (Compound **9**) as a yellow oil.

1.13 Synthesis of 2-(2'-Propenyl)-1-(2'-propenoxy)-3,3-diphenylindane (Compound **6**)

2-(2'-Propenyl)-1-(2'-propenoxy)-3,3-diphenylindane (Compound **6**) was synthesized as follows: 3,3-diphenylindanone (Compound **2**) 2.0 g (0.007 mole) and 0.28 g (0.0084 mole) sodium hydride were stirred at room temperature in 40 mL of dimethylformamide for 1 hour. The reaction mixture was then added drop-wise to 0.64 mL (0.0078 mole) of allyl bromide at -50 °C. The mixture was then warmed to reflux and refluxed for 1 hour. After cooling to room temperature, 50 mL of water was added. The mixture was extracted with ethyl acetate, dried over sodium sulfate and concentrated *in vacuo*. 2-(2'-propenyl)-1-(2'-propenoxy)-3,3-diphenylindane (Compound **6**) was isolated in 30% yield as the first fraction from a silica gel column using 10% dichloromethane in hexane as eluate.

1.14 Synthesis of 1-Acetoxy-3,3-diphenylindane (Compound **7**)

1-Acetoxy-3,3-diphenylindane (Compound **7**) was synthesized as follows: 1-Hydroxy-3,3-diphenylindane (Compound **3**) (0.06 g, 0.0021 mol) was combined with 0.3 mL (0.0022 mol) triethylamine in 10 mL of dichloromethane. The mixture was warmed to reflux with stirring to dissolve all of the starting material. The heat was removed and 0.16 mL (0.0022 mol) of acetyl chloride was added to the warm solution. The mixture was returned to reflux and stirred at reflux for 1 h. After cooling to room temperature, the reaction was

quenched by adding 5 mL of water. The reaction mixture was extracted with dichloromethane and the organic layer dried over sodium sulfate. Evaporation of the solvent gave 0.008 g (11% yield) of 1-acetoxy-3,3-diphenylindanone (Compound 7) as an off-white solid with a melting point of 90°C.

1.15 Synthesis of 6-Chloro-3,3-di(4-chlorophenyl)indanone (Compound 8)

6-Chloro-3,3-di(4-chlorophenyl)indanone (Compound **8**) was synthesized as follows: 3,3,3-Tris(4-chlorophenyl) propionic acid (1.5 g, 0.004 mol) was stirred in 10 mL of concentrated sulfuric acid at room temperature for 1.5 h. The reaction mixture was then poured into 10 mL of ice water and the mixture extracted with dichloromethane. The solvent was evaporated and 0.8 g (54% yield) of 6-Chloro-3,3-di(4-chlorophenyl)indanone (Compound **8**) was collected as an off-white solid having a melting point of 134°C.

1.16 Synthesis of 6-Chloro-2-cyanomethyl-3,3-di(4'-chlorophenyl)indanone (Compound 10)

6-Chloro-2-cyanomethyl-3,3-di(4'-chlorophenyl)indanone (Compound **10**) was synthesized as follows: 6-Chloro-3,3-di(4'-chlorophenyl)indanone (Compound **8**) (1.0 g, 0.0026 mol) was dissolved in 5 mL of tetrahydrofuran and 0.124 g (0.0031 mol) of sodium hydride was added. The reaction mixture was stirred at room temperature for 1.5 h before 0.22 mL (0.0215 mol) of bromoacetonitrile was added. After stirring overnight the reaction was quenched with water and extracted with ethyl acetate. The extracts were combined and the solvent removed *in vacuo*. The residue was purified on a silica gel column using 5% ethyl acetate in hexane as the eluent. The first fraction from the column was recovered starting material (1.05 g). The second fraction contained undesired side reaction product. The third fraction contained the desired product. After evaporation of the solvent, 0.179 g (16% yield) 6-Chloro-2-cyanomethyl-3,3-di(4'-chlorophenyl)indanone (Compound **10**) as a pale yellow solid was obtained.

1.17 Synthesis of 6-Chloro-3,3-di(4'-chlorophenyl)-2-N-oxime-3,3-diphenylindane (Compound 11)

6-Chloro-3,3-di(4'-chlorophenyl)-2-N-oxime-3,3-diphenylindane (Compound **11**) was synthesized as follows: 6-Chloro-3,3-di(4'-chlorophenyl)indanone (compound 8) (0.80 g, 0.0021 mol) was combined with 0.72 g (0.0103 mol) of hydroxylamine hydrochloride and

1.69 g (0.0206 mol) of sodium acetate and dissolved in 25 mL of methanol. The solution was stirred at room temperature for 16 h and then water was added. The mixture was extracted with ethyl acetate and the organic layer was dried over magnesium sulfate. Evaporation of the solvent gave 0.85 g (100% yield) of 6-Chloro-3,3-di(4'-chlorophenyl)-2-N-oxime-3,3-diphenylindane (Compound **11**) as a white solid having a melting point of 85°C.

1.18 Synthesis of 2-Acetamide-3,3-diphenylindanone (Compound **12**)

2-Acetamide-3,3-diphenylindanone (Compound **12**) was synthesized as follows: 2-Cyanomethyl-3,3-diphenylindanone (0.685 g, 0.0021 mol) was combined with 10 mL of concentrated sulfuric acid and 10 mL of glacial acetic acid. The solution was stirred at room temperature for 3 h and then water was added. The mixture was cooled in an ice bath and neutralized to pH 7 with concentrated ammonium hydroxide and then extracted with ethyl acetate. The organic layer was dried over magnesium sulfate. Evaporation of the solvent gave 0.77 g of a light orange solid. This solid was crystallized from a mixture of ethyl acetate and hexane. 2-Acetamide-3,3-diphenylindanone (Compound **12**) was obtained as off-white crystals, 0.527g (73% yield), having a melting point of 169 - 171°C.

1.19 Synthesis of 2-Cyanomethyl-3,3-diphenylindanol (Compound **13**)

2-Cyanomethyl-3,3-diphenylindanol (Compound **13**) was synthesized as follows: 2-Cyanomethyl-3,3-diphenylindanone (Compound **2**) (0.311 g, 0.001 mol) was dissolved in 5 mL of ethanol at room temperature. Sodium borohydride (0.437 g, 0.011 mol) was added and the mixture was stirred at room temperature for 15 min. The mixture was diluted with ethyl acetate and the pH was adjusted to 2 with 2N hydrochloric acid. The layers were separated and the aqueous layer extracted twice with ethyl acetate. The combined extracts were evaporated *in vacuo* and the crude product was purified on a silica gel column using 20% ethyl acetate in hexane. The first fraction was unreacted starting material. The second fraction, when the solvent was evaporated, gave 0.16 g (51% yield) of 2-Cyanomethyl-3,3-diphenylindanol (Compound **13**) as a white solid having a melting point of 79 - 85°C.

1.20 Synthesis of 2-Acetamide-3,3-diphenylindanol (Compound **14**)

2-Acetamide-3,3-diphenylindanol (Compound **14**) was synthesized as follows: 2-Acetamide-3,3-diphenylindanone (Compound **12**) (0.100 g, 0.0003 mol) was dissolved in 2

mL of ethanol and 0.5 mL of methanol at room temperature. Sodium borohydride (0.136 g, 0.0004 mol) was added and the mixture was stirred at room temperature for 3 hours. The mixture was quenched with 2N hydrochloric acid to pH 1. The mixture was extracted with ethyl acetate and the combined extracts dried over magnesium sulfate. Evaporation of the solvent gave an off-white solid which was crystallized from a mixture of ethyl acetate/hexane. 2-Acetamide-3,3-diphenylindanol (Compound **14**) was collected by filtration as a white solid (0.026 g, 25% yield) having a melting point of 218 - 220°C.

1.21 Synthesis of 3,3-Diphenylindanone-2-methyl acetate (Compound **15**)

3,3-Diphenylindanone-2-methyl acetate (Compound **15**) was synthesized as follows: 3,3-Diphenylindanone (Compound **2**) (3.84 g, 0.0135 mol) was dissolved in 30 mL of tetrahydrofuran at room temperature. Potassium hydride (1.85 g, 0.0162 mol) was added and the mixture was stirred at room temperature for 1 hour. Methyl chloroformate (1.25 mL, 0.0162 mol) was added and the mixture was stirred at room temperature for 1 hour. The mixture was quenched with water and extracted with ethyl acetate. The combined extracts were dried over magnesium sulfate. Evaporation of the solvent gave an dark brown solid which was purified on a silica gel column using 5% ethyl acetate in hexane as eluent. The product was collected in the second fraction off the column. Evaporation of the solvent gave a slightly wet, pink solid which was stirred in hexane. 3,3-Diphenylindanone-2-methyl acetate (Compound **15**) was collected by filtration as an off-white solid (2.06 g, 45% yield) having a melting point of 140 - 142°C.

1.22 Synthesis of 3,3-Diphenyl-1-indanyl 2-naphthylmethyl ether (Compound **16**)

3,3-Diphenyl-1-indanyl 2-naphthylmethyl ether (Compound **16**) was synthesized as follows: 1-Hydroxy-3,3-diphenylindane (Compound **3**) (0.25 g, 0.87 mmol) was dissolved in 10 mL of dimethylformamide and cooled to 0°C with stirring. Sodium amide (0.042 g, 1.04 mmol) was added and the reaction stirred for 0.5 h at 0°C before 0.23 g (1.04 mmol) of 2-bromomethylnaphthalene was added. The reaction mixture was allowed to warm to room temperature and stirred for 15h. An equal volume of water was added to the mixture and this was extracted twice with 50 mL of ethyl acetate. After drying over magnesium sulfate the solvent was evaporated and the resultant solid was purified on a silica gel column using 2% ethyl acetate in hexane as the eluent. The second fraction collected was the desired product.

Evaporation of the solvent gave 0.300 g (81% yield) of 3,3-Diphenyl-1-indanyl 2-naphthylmethyl ether (Compound **16**) as an off-white, sticky solid.

1.23 Synthesis of 3,3-Diphenyl-1-indanyl α-(4-methyltoluate) ether (Compound **17**)

3,3-Diphenyl-1-indanyl α-(4-methyltoluate) ether (Compound **17**) was synthesized as follows: 1-Hydroxy-3,3-diphenylindane (Compound **3**) (0.505 g, 1.8 mmol) was combined with 0.069 g (2.9 mmol) of sodium amide in 10 mL of dimethylformamide and stirred at room temperature for 1.5 h before 0.667 g (2.9 mmol) of methyl 4-(bromomethyl)benzoate was added. The reaction mixture was stirred for 18h. The reaction mixture was poured into 50 mL of water and extracted four times with 25 mL of ethyl acetate. The combined extracts were washed with brine, dried over sodium sulfate and the solvent evaporated to yield a yellow oil. The oil was purified by vacuum distillation to give 0.370 g (47% yield) of 3,3-Diphenyl-1-indanyl α-(4-methyltouate) ether (Compound **17**) as a yellow solid having a melting point of 50-52°C.

1.24 Synthesis of 3,3-Diphenyl-1-indanyl α-(2-chlorotoluyl) ether (Compound **18**)

3,3-Diphenyl-1-indanyl α-(2-chlorotoluyl) ether (Compound **18**) was synthesized as follows: 1-Hydroxy-3,3-diphenylindane (Compound **3**) (0.503 g, 1.8 mmol) was combined with 0.075 g (3.1 mmol) of sodium amide in 10 mL of dimethylformamide and stirred at room temperature for 1.5 h before 0.40 mL (3.2 mmol) of 2-chlorobenzyl chloride was added. The reaction mixture was stirred for 21h. The reaction mixture was poured into 50 mL of water and extracted four times with 25 mL of ethyl acetate. The combined extracts were washed with brine, dried over sodium sulfate and the solvent evaporated to yield a yellow oil. The oil was purified by vacuum distillation to give 0.520 g (70% yield) of 3,3-Diphenyl-1-indanyl α-(2-chlorotoluyl) ether (Compound **18**) as a solid having a melting point of 27 - 29°C.

1.25 Synthesis of 3-(3',3'-diphenyl-2'-indanyl-1'-one)propanol (Compound **19**)

3-(3',3'-diphenyl-2'-indanyl-1'-one)propanol (Compound **19**) was synthesized as follows: 3,3-Diphenylindanone (Compound **2**) (2 g, 0.007 mol) was dissolved in 10 mL of tetrahydrofuran, cooled in an ice bath, and 0.97 g (0.0085 mol) of potassium hydride was added. The reaction mixture was stirred at room temperature for 0.5 h before 0.72 mL (0.0077 mol) of 3-bromo-1-propanol was added. After stirring overnight the reaction was

quenched with water and extracted with ethyl acetate. The combined extracts were dried over magnesium sulfate and the solvent removed *in vacuo*. The residue was purified on a silica gel column using 15% ethyl acetate in hexane as the eluent. The first fraction from the column was recovered starting material (1.05 g). The second fraction contained the product. After evaporation of the solvent, 0.84 g (35% yield) of 3-(3',3'-diphenyl-2'-indanyl-1'-one)propanol (Compound **19**) as a beige solid having a melting point of 98°C was obtained.

1.26 Synthesis of 2-(Ethyl-2'-(1,3-dioxolane))-1-hydroxy-3,3-diphenylindene (Compound **20**)

2-(Ethyl-2'-(1,3-dioxolane))-1-hydroxy-3,3-diphenylindene (Compound **20**) was synthesized as follows: 3,3-Diphenylindanone (Compound **2**) (4.0 g, 0.0141 mol) was dissolved in 30 mL of tetrahydrofuran at room temperature. Potassium hydride (2.4 g, 0.0175 mol) was added and the mixture was stirred at room temperature for 0.5 h. 2-(2-Bromoethyl)-1,3-dioxolane (2.0 mL, 0.0170 mol) was added and the mixture was continued stirring overnight at room temperature. The mixture was quenched with water and extracted with ethyl acetate. The combined extracts were purified on a silica gel column using 8% ethyl acetate in hexane followed by 10% ethyl acetate in hexane as eluent. The product was collected in the second fraction off the column. Evaporation of the solvent gave 2-(Ethyl-2'-(1,3-dioxolane))-1-hydroxy-3,3-diphenylindene (Compound **20**) as an off-white solid (0.47 g, 9% yield) having a melting point of 124-126°C.

1.27 Other Compounds

Other compounds of the invention can be synthesized by routine modification of the above-described syntheses, or by other methods that are well known in the art. Compound **1** is available from Maybridge Chemical Company (distributor: Ryan Scientific, South Carolina).

Example 2. *In Vitro* Activity

This Example demonstrates the ability of several exemplary compounds of structural formula (I) to inhibit the Gardos channel of erythrocytes (Gardos Channel Assay) and/or mitogen-induced cell proliferation (Mitogenic Assay) *in vitro*. The assays are generally applicable for demonstrating the *in vitro* activity of other compounds of structural formula (I).

Methods. The percent inhibition of the Gardos channel (10 μM compound) and the IC_{50} were determined as described in Brugnara *et al.*, 1993, J. Biol. Chem. 268(12):8760-8768. The percent inhibition of mitogen-induced cell proliferation (10 μM compound) and the IC_{50} were determined or described in Benzaquen *et al.* (1995, Nature Medicine 1:534-540) with NIH 3T3 mouse fibroblast cells (ATCC No. CRL 1658). Other cell lines, *e.g.*, cancer cells, endothelial cells and fibroblasts, as well as many others, may be used in the cell proliferation assay. Selection of a particular cell line will depend in part on the desired application, and is well within the capabilities of an ordinarily skilled artisan.

Results. The results of the experiment are provided in Table 2, below. Clotrimazole is reported for purposes of comparison. Most of the compounds tested exhibited significant activity in both assays. All of the compounds tested exhibited significant activity in at least one of the assays.

Table 2

Pharmacological Activities of Various Compounds
(% Inhibition measured at 10 μM)

Compound Number	Mitogenic Assay		Gardos Channel Assay	
	IC_{50} (μM)	Inhibition (%)	IC_{50} (μM)	Inhibition (%)
Clotrimazole	0.626	93.0	0.046	99.3
(1)	0.700	97.0	0.419	98.0
(2)	1.300	99.0	1.006	100.0
(3)	1.100	90.0	0.819	100.0
(4)	2.600	99.0	1.350	100.0
(5)	--	29.0	--	67.3
(6)	3.400	90.0	--	35.0
(7)	3.400	98.0	1.152	88.0
(8)	2.000	97.0	0.176	30.0
(9)	--	45.0	0.505	100.0
(10)	3.300	98.0	--	49.5
(11)	3.400	99.0	--	50.0
(12)	--	31.0	0.189	99.5

Compound Number	Mitogenic Assay		Gardos Channel Assay	
	IC_{50} (μM)	Inhibition (%)	IC_{50} (μM)	Inhibition (%)
(13)	--	12.0	1.590	99.5
(14)	--	3.0	2.961	90.5
(15)	7.500	80.0	2.901	54.8
(16)	--	75.0	--	0
(17)	--	76.0	--	0
(18)	--	73.0	--	0
(19)	1.500	99.0	5.952	43.7
(20)	--	81.0	--	0

Example 3. Activity In Cancer Cell Lines

This Example demonstrates the antiproliferative effect of several exemplary compounds of formula (I) against a variety of cancer cell lines. The assays are generally applicable for demonstrating the antiproliferative activity of other compounds of formula (I).

Methods. Growth of Cells The antiproliferative assays described herein were performed using standard aseptic procedures and universal precautions for the use of tissues. Cells were propagated using RPMI 1640 media (Gibco) containing 2% N 5% fetal calf serum (Biowhittaker) at 37°C, 5% CO_2 and 95% humidity. The cells were passaged using Trypsin (Gibco). Prior to addition of test compound, the cells were harvested, the cell number counted and seeded at 10,000 cells/well in 100 μl 5% fetal calf serum (FCS) containing RPMI medium in 96-well plates and incubated overnight at 37°C, 5% CO_2 and 95% humidity.

On the day of the treatment, stock solutions of the test compounds (10 mM compound/DMSO) were added in 100 μl FCS containing medium to a final concentration of 10-0.125 μM and the cells were incubated for 2, 3 or 5 days at 37°C, 5% CO_2 and 95% humidity.

Following incubation, the cellular protein was determined with the ulforhodamine B (SRB) assay (Skehan P et al., 1990, J. Natl. Cancer Inst. 82:1107-1112). Growth inhibition, reported as the concentration of test compound which inhibited 50% of cell proliferation (IC_{50}) was determined by curve fitting.

Values for VP-16, a standard anti-cancer agent, are provided for comparison.

Except for MMRU cells, all cancer cell lines tested were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The ATCC assession numbers were as follows: HeLa (CCL-2); CaSki (CRL-1550); MDA-MB-231 (HTB-26); MCF-7 (HTB-22); A549 (CCL-185); HTB-174 (HTB-174); HEPG2 (HB-8065); DU-145 (HTB-81); SK-MEL-28 (HTB-72); HT-29 (HTB-38); HCT-15 (CCL-225); ACHN (CRL-1611); U-118MG (HTB-15); SK-OV-3 (HTB-77).

MMRU cells (Stender et al., 1993, J. Dermatology 20:611-617) were a gift of one of the authors.

Results. The results of the cell culture assays are presented in Tables 3 and 4, below.

Table 3

SRB ASSAY RESULTS
(5% FCS, 5 Day Incubation)

Cancer Type	Cell Line	Test Compound IC_{50} (μM)		
		VP-16	8	11
Cervical	HeLa	<1.25	>10	5.1
	CaSki	1.8	6.8	7
Breast	MDA-MB-23	<1.25	>10	>10
	MCF7	<1.25	5.5	4.4
Lung	A549	<1.25	8.9	8.8
	HTB174	<1.25	>10	5.9
Hepatocel	HEPG2	<1.25	6.4	5.8
Prostate	DU-145	<1.25	>10	>10
Melanoma	SK-MEL-28	<1.25	>10	5.5
	MMRU	<1.25	>10	6.2
Colon	HT29	<1.25	8.3	6.8
	HCT-15	1.3	>10	6.6
Renal	ACHN	<1.25	>10	>10
CNS	U118MG	2.2	>10	>10

Ovary	SK-OV-3			>10
Normal	HUVEC	<1.25	>10	6.4
human	GM	1.4	>10	>10
	3T3		>10	>10
mouse	L929	<1.25	>10	8.6

Table 4

SRB RESULTS

Compound	Conditions %FCS/days	Test Compound IC_{50} (μM) in Various Cell Lines					
		A549	HT29	MMRU	MCF7	HEPG2	U118MG
VP-16	2%/3 days	2.3	20	<2.5	<2.5		
3	5%/2 days	>10	>10	5.8			
4	2%/3 days	8.5	<2.5	8.2	<2.5		
8	5%/3 days	>10	>10	3.3	>10	7.8	>10

Example 4. Formulations

The following examples provide exemplary, not limiting, formulations for administering the compounds of the invention to mammalian, especially human, subjects. Any of the compounds described herein, or pharmaceutical salts or hydrates thereof, may be formulated as provided in the following examples.

4.1 Tablet Formulation

Tablets each containing 60 mg of active ingredient are made up as follows:

Active Compound	150 mg
Starch	150 mg
Microcrystalline Cellulose	150 mg
Sodium carboxymethyl starch	4.5 mg
Talc	1 mg
Polyvinylpyrrolidone (10% in water)	4 mg
Magnesium Stearate	0.5 mg
	160 mg

The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S.

sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules are dried at 50°-60°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules, which, after mixing are compressed by a tablet machine to yield tablets each weighing 150 mg.

Tablets can be prepared from the ingredients listed by wet granulation followed by compression.

4.2 Gelatin Capsules

Hard gelatin capsules are prepared using the following ingredients:

Active Compound	250 mg/capsule
Starch dried	200 mg/capsule
Magnesium Stearate	10 mg/capsule

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

4.3 Aerosol Solution

An aerosol solution is prepared containing the following components:

Active Compound	0.25% (w/w)
Ethanol	29.75% (w/w)
Propellant 22 (Chlorodifluoromethane)	77.00% (w/w)

The active compound is mixed with ethanol and the mixture added to a portion of the propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

4.4 Suppositories

Suppositories each containing 225 mg of active ingredient are made as follows:

Active Compound	225 mg
Saturated fatty acid glycerides	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

4.5 Suspensions

Suspensions each containing 50 mg of medicament per 5 mL dose are made as follows:

Active Compound	50 mg
Sodium carboxymethylcellulose	50 mg
Syrup	1.25 mL
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to	5 mL

The active ingredient is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor and some color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

Example 5: Clotrimazole inhibits water and electrolyte secretion in intestinal epithelial cells.

The biochemical basis of secretory diarrhea involves intestinal Cl^- secretion in intestinal crypt cells. Under normal conditions, Cl^- ions are maintained within intestinal crypt cells at levels above their electrochemical potential by primarily and secondarily active transport mechanisms such as the Na/K ATPase pumps and Na/K/2Cl cotransporters. Cl^- is transported into the lumen from the intestinal crypt cells through apical Cl^- channels. Intracellular levels of K^+, cAMP, cGMP, and Ca^{++} are all involved in regulating the secretory response.

T84 cells were used to determine whether clotrimazole regulates Cl^- secretion in intestinal crypt cells. T84 cells form confluent monolayers of columnar epithelia that exhibit high transepithelial resistances, polarized apical and basilateral membranes, and cAMP and Ca^{++} regulated Cl^- secretory pathways analogous to those found in native intestine.

Methods. Growth of T84 cells. T84 cells obtained from ATCC were cultured and passaged in equal parts of dulbecco's modified eagle's medium (DMEM), 1g/1D-glucose) and Hams F-12 nutrient mixture, supplemented with 5% newborn calf serum, 15 mM HEPES, 14

mM Na HCO_3, 40mg/l penicillin, 8mg/l ampicillin, 0.90 mg/l streptomycin. Cells were seeded at confluent density onto 0.33 cm^2 or 5cm^2 Transwell inserts (Costar, Cambridge, MA) coated with dilute rat collagen solution as previously described (Lencer et al., J. Clin. Invest., 92: 2941-2951 (1993); Lencer et al., J. Cell Biol. 117: 1197-1209 (1992). Transepithelial resistances attain stable levels (>1000 Ohms.cm^2) after 7 days. The development of high transepithelial resistances correlated with the formation of confluent monolayers with well-developed tight junctions as assessed by morphological analysis, and with the ability of monolayers to secrete Cl^- (Madara et al., Gastro. 92: 1133-1145 (1987).

Electrophysiology (mesurement of electrogenic Cl^- secretion). Confluent monolayers were transferred to Hanks Buffered Salt Solution (HBSS) containing 0.185 g/l $CaCl_2$, 0.098 g/l $MgSO_4$, 0.4 g/l KCl, 0.06 g/l KH_2PO_4, 8 NaCl, 0.048 g/l Na_2HPO_4, 1 g/l glucose, and 10nM HEPES, pH 7.4. Serosal and mucosal reservoirs were interfaced with Calomel and Ag-Ag Cl electrodes via 5% agar bridges made with Ringer's buffer. Transepithelial resistance was measured using a dual voltage clamp device to apply 25 or 50μA current pulses. Short circuit current (ISC) was calculated using Ohms law as previously described (Lencer et al., J. Clin. Invest. 92: 2941-2951 (1993); Lencer et al. J. Cell Biol. 117: 1197-1209 (1992).

Results. Clotrimazole reversibly inhibits Cl^- secretion elicited by Ca^{++}- or cAMP-dependant agonists in T84 cells. Previous studies have shown that Cl^- secretion in T84 cells is controlled by K^+ efflux pathways which are biophysically and pharmacologically distinct from one another. One pathway participates in the secretory response to cAMP-dependent agonists and displays sensitivity to Ba^{++} salts (McRoberts, et al., *J. Biol. Chem.* 260: 14163-14172 (1985); Reenstra, *Am J. Physiol.* 264: C161-168 (1993)). The other mediates the response to Ca^{++}-dependent agonists, and is Ba^{++}-insensitive. Several pathway specific agonists of K+ channels are useful for determining whether a particular compound is functioning through a cAMP or Ca^{++} specific pathway. For instance, vasoactive intestinal peptide (VIP) and cholera toxin are cAMP mediated agonists of the K^+ channel, whereas, carbachol is a Ca^{++}-dependent agonist of the Ca^{++} regulated K^+ channels. The pathway by which a particular inhibitor of Cl^- secretion in T84 cells is functioning may be identified by measuring the ability of the inhibitor to modify transepithelial resistances in T84 cells which have been treated with VIP or carbachol to stimulate Cl^- secretion.

T84 cells were grown as described above and Cl^- secretion was stimulated by the addition to the media of either carbachol (100mM) or VIP (5nM). The cells were then treated

with BaCl (3mM), charybdotoxin (100nM), or clotrimazole (33mM). The short circuit current (ISC) was determined for the various inhibitor treatments as a percentage of the control in the absence of inhibitor (Fig. 3). BaCl strongly inhibited the secretory response to the cAMP mediated agonist VIP, but had no apparent affect on the secretory response elicited by the Ca^{++}-dependent agonist carbachol. In contrast, the scorpion venom Charybdotoxin strongly inhibited the secretory response elicited by carbachol, but had minimal affects on Cl^- secretion elicited by VIP. However, clotrimazole inhibited the Cl^- secretory responses to both agonists. Inhibition of Cl- secretion by clotrimazole was fully reversible (96±2%, n = 4) after 60 min recovery in the presence of 0.01 mg/ml bovine serum albumin.

To examine possible effects of clotrimazole on the synergy between cAMP and Ca^{++}-mediated agonists, monolayers, initially stimulated with VIP were allowed to reach steady-state levels of secretion and then additionally exposed to carbachol (100 μM). Clotrimazole was slightly more effective in inhibiting the secretory response to carbachol than to cAMP with IC50 values of 3 and 8 μM, respectively. When the effects of clotrimazole on cAMP- and Ca^{++}-dependent secretory pathways were examined on the same monolayers., inhibition of the synergistic response to VIP plus carbachol was found to parallel the inhibition of secretion promoted by Ca^{++} agonists alone. In low doses (=10^{-7} or less), clotrimazole potentiated slightly (by 5-10%) the Cl-secretory responses to either agonist. clotrimazole inhibited effectively the secretory response to cholera toxin (20 nM, a cAMP-dependent agonist) and *E. Coli* heat-stabile toxin (100 nm, a cGMP-agonist) (IC50 values of 10 μM and 15 μM, respectively).

The effect of clotrimazole on K^+ conductances was also examined by isotopic flux studies using ^{86}RB. T84 cells were grown in the presence of a cAMP agonist, VIP, or a Ca^{++} mediated agonist (Thapsigargin). Clotrimazole was added and ^{86}RB efflux was measured. Clotrimazole significantly inhibited baseline and Ca^{++} stimulated ^{86}RB efflux in the presence of both cAMP and Ca^{++} mediated agonists compared to those cells which were not treated with clotrimazole.

Other aromatic compunds of the invention were found to inhibit chloride secretion. Although clotrimazole was the most potent inhibitor tested of cAMP and Ca^{++} elicited Cl secretion, ketoconazole, econazole, miconazole, and 2-chlorophenyl-bis-phenyl methanol also were effective at inhibiting chloride secretion.

Taken together, these studies indicate that clotrimazole inhibits Cl^- secretion elicited

by cAMP or Ca^{++} mediated K^+ channels in T84 cells .

Example 6: Clotrimazole acts at distal steps in the cAMP and Ca++-dependent signal transduction pathways.

To determine the site of clotrimazole action, the effects of clotrimazole pretreatment were examined on monolayers stimulated with agonists that initiate Cl- secretion at sequential steps in the cAMP signalling cascade. T84 monolayers were preincubated in HBSS in the presence or absence of clotrimazole (33 μM) and then stimulated with either 5 μM VIP (which activates adenylate cyclase through heterotrimeric GTPase-linked cell surface receptors), 10 μM forskolin (which activates adenylate cyclase directly), or 3 mM 8Br-cAMP (a direct stimulator of protein kinase A). Clotrimazole inhibited the secretory response to each of these agonists. These data provide evidence that clotrimazole acts at a step distal to the activation of Protein Kinase A.

Ca^{++}-dependent intracellular signaling in T84 and other non-exciteable cells involves recruitment of inositol trisphosphate (IP3)-dependent intracellular Ca++ stores (Halm and Frizzell, *Textbook of Secretory Diarrhea*, Raven Press, 47-58 (1990); Mandel et al., *J. Biol. Chem.* 267: 704-712 (1986); Halm et al., *Am. J. Physiol. (Cell Physiol. 23)* 254:C505-C511 (1988)), and subsequent activation of plasma membrane Ca++ influx pathways (Barrett, *Am. J. Physiol. (Cell Physiol. 34)*: C859-C868 (1993)). Downstream events may be mediated by [Ca++]i, IP3, diacylglycerol, or as yet unidentified diffusable factors (Putney and Bird, *Cell* 75:199-201 (1993)). To examine the site of clotrimazole action alone, this signalling, cascade, T84 monolayers pretreated in the presence or absence of clotrimazole (33 μM) were stimulated with the Ca++-dependent agonists carbachol (100 μM which elicits both Ca++ and IP_3 signals), thapsigargin (5 μM, which elevates cytoplasmic Ca++ via inhibition of ER Ca++-ATPase) (Vandorpe et al., *Biophys. J.* 66:46-58 (1994)), or the Ca++ ionophore ionomycin (10 μM). Clotrimazole inhibited strongly the Cl-secretory response to each to these reagents. These data suggest that clotrimazole acts at steps in the secretory response distal to the release of intracellular Ca++ stores.

Example 7: Clotrimazole does not affect apical membrane anion conductance or basolateral NaK2Cl cotransporters.

Methods. ^{125}I Efflux Studies Confluent monolayers on 5 cm^2 Transwell inserts were

used 10-14 days after plating. ^{125}I was measured as an indicator of apical Cl-, channel and basolateral K+ channel activity as previously described (Venglarik, et al, *Am. J. Physiol. (Cell Physiol. 28)*:C358-C364 (1990). Monolayers were preincubated at 37° C with 4 μCi/ml ^{125}I in HBSS for 90 minutes, with 33 μM clotrimazole absent or present during the final 30 minutes of this 90 min preincubation period. Clotrimazole pretreatment did not alter ^{125}I loading of the cells. After washing twice in fresh HBSS, 0.5 ml samples were obtained every two min from the apical reservoir and replaced with fresh HBSS. After four baseline samples were obtained, the cells were treated (at t = 8 minutes) with vasoactive intestinal peptide (VIP, 5 μnM) or thapsigargin (5 μM) to stimulate Cl- secretion, and an additional 15 timed samples were obtained. Finally, the cell monolayer was rinsed, cut with its support from the polystyrene ring, and the residual cell-associated radioactivity was determined. Monolayers were maintained at 37° C in room air throughout the study. ^{125}I was counted by gamma counting and normalized to percent total uptake as previously described (Venglarik, et al, *Am. J. Physiol. (Cell Physiol. 28)*:C358-C364 (1990).

^{86}Rb Uptake Studies Confluent monolayers on 5 cm^2 Transwell inserts were incubated for 30 minutes in HBSS at 37° C. A group of control and CLT treated (33 μM, for 30 min) monolayers were treated with bumetanide (10 μM for 12 min). All monolayers were then treated with VIP (5nM and shifted to HBSS containing 1 μCi/ml ^{86}Rb for 3 minutes at 37° C. ^{86}Rb uptake was terminated by washing the inserts in an ice-cold solution containing 100mM $MgCl_2$, and 10mM TRIS-CL, pH 7.4. Monolayers were cut from their inserts, placed into scintillation vials, and counted using standard methods.

Results. Studies were conducted to determine whether the inhibition of electrogenic Cl- secretion might occur by blockade of apical membrane Cl-channels, or blockade of basolaterally situated NaK2Cl cotransporters. To determine if clotrimazole affected ion conductance through apical membrane Cl-channels, we examined the time course of ^{125}I efflux from T84 monolayers pretreated in the presence or absence of clotrimazole (Venglarik, et al, *Am. J. Physiol. (Cell Physiol. 28)*:C358-C364 (1990). Clotrimazole had little or no effect on the time course of ^{125}I efflux from monolayers treated with VIP. Rate constants for 125I efflux from monolayers treated or not treated with clotrimazole were indistinguishable (0.0637 vs.0.0645 % uptake/minute, n=2 in duplicate). Clotrimazole had similar lack of effect on ^{125}I efflux stimulated by thapsigargin.

We next tested the effect of clotrimazole on basolateral NaK2Cl cotransporters, as

assessed by bumetanide-sensitive ^{86}Rb uptake (Matthews et al., *J. Biol. Chem.* 269:15703-15709 (1994)). Clotrimazole treatment reduced the total amount of ^{86}Rb uptake by 53.6±5.8% (mean±SEM. n=6), but had no effect on the fractional component that was bumetanide-sensitive (88±3.2 vs 75.2 ±12.7% total uptake, mean±SEM). Taken together, these data strongly suggest that clotrimazole does not affect Cl- secretion in T84 cells via inhibition of either apical membrane Cl- channels or basolateral membrane NaK2Cl cotransporters.

Example 8: Clotrimazole inhibits Chloride secretion by inhibiting K+ efflux through basolateral K+ channels in T84 cells.

8.1 Clotrimazole inhibits chloride secretion by blockade of K+ transport through both Ba++-sensitive and charybdotoxin-sensitive channels

Methods. ^{86}Rb Efflux Studies Confluent monolayers on 5 cm^2 Transwell inserts were used 10-14 days after plating. ^{86}Rb flux was measured as an indicator of apical Cl-, channel and basolateral K+ channel activity as previously described (Venglarik, et al, *Am. J. Physiol. (Cell Physiol. 28)*:C358-C364 (1990). Monolayers were preincubated at 37° C with 4 μCi/ml ^{86}Rb in HBSS for 90 minutes, with 33 μM clotrimazole absent or present during the final 30 minutes of this 90 min preincubation period. clotrimazole pretreatment did not alter ^{86}Rb loading of the cells. One ml samples were obtained and replaced from the basolateral reservoir. After four baseline samples were obtained, the cells were treated (at t = 8 minutes) with vasoactive intestinal peptide (VIP, 5 μnM) or thapsigargin (5 μM) to stimulate Cl-secretion, and an additional 15 timed samples were obtained. Finally, the cell monolayer was rinsed, cut with its support from the polystyrene ring, and the residual cell-associated radioactivity was determined. Monolayers were maintained at 37° C in room air throughout the study. ^{86}Rb was counted by scintillation counting and normalized to percent total uptake as previously described (Venglarik, et al, *Am. J. Physiol. (Cell Physiol. 28)*:C358-C364 (1990).

Results. K+ channel activity was estimated by measurement of ^{86}Rb efflux. Clotrimazole was found to significantly inhibit the rate of ^{86}Rb efflux after treatment with the cAMP agonist VIP (5 μM). The rate constant for VIP-stimulated ^{86}Rb efflux was reduced by 87% in monolayers treated with clotrimazole (0.0062 vs. 0.0465 % uptake/minute, n=2 in triplicate). clotrimazole inhibited to a similar degree ^{86}Rb efflux from monolayers stimulated

with thapsigargin (panel B, rate constants 0.011 vs. 0.048% uptake/minute, n=2), suggesting that clotrimazole can inhibit Cl- secretion by blockade of K+ transport through both Ba++-sensitive and charybdotoxin-sensitive channels.

8.2. Clotrimazole inhibits chloride secretion through distinct cAMP and Ca^{++} sensitive basolateral K^+ channels

Methods. Selective mebrane Permeabilization and Measurement of Potassium Conductance of the Basolateral Membrane. The basolateral potassium conductance was measured using the technique developed by Dawson and co-workers. A potassium gradient (mucosal to serosal) was first established across the monolayer using asymmetric mucosal and serosal buffers containing K^+ as the sole permeant ion. The addition of amphotericin B (20 μM) to the mucosal reservoir forms conductive pores in the apical membrane, and thus removes all resistance to transepithelial potassium movement across this membrane. Thus, under the conditions of the experiment, in which the monolayer is short circuited (i.e., voltage-clamped at zero potential) and the transepithehal potassium gradient is constant, the amphotericin-dependent Isc becomes a measure of the rate of the transepithelial potassium flux across basolateral membranes. Changes in short circuit current (Isc), then represent changes in basolateral K^+ conductances (gK). Isc and K^+ conductances were measured using calomel electrodes, 3M KCI-agar bridges, and a voltage clamp (University of Iowa, Iowa City). To generate a voltage-current channel relationships, currents were elicited by 1 sec test potentials from -80 to +80 in 10 mV increments in the asymmetrical high K^+ gluconate solution.

Calculation of basolateral membrane K^+ permeability. Membrane permeabilities were calculated according to the formula:

$$^{P}K = (cm/s) = ^{J}K\ (mM/cm^2 \cdot s)/\Delta[K^+]\ (mM/cm^3)$$

where Δ $[K^+]$ is equal to the difference in K^+ concentration (135 mM) between the asymmetric apical and basolateral bathing solutions. Maximal Isc values were converted into K^+ fluxes by dividing by the Faraday constant F (96,500 coulombs/mol) as previously described (Huflejt et al., *J. Clin. Invest.* 93: 1900-1910 (1994)).

Results. Basolateral K+ transport was examined in T84 monolayers permeabilized apically by pretreatment with amphotericin B. Apical and basolateral buffers contained K+ as the sole permeant ion. All studies were performed with a 135 mM basolaterally directed K+

gradient. This method has been utilized previously to examine both Cl- and K+ transport in T84 cells and HT29-Cl.16E cells. Briefly, ion conductances in the luminal or basolateral membranes of confluent T84 cell monolayers can be assessed separately by selectively permeabilizing the apical or basolateral membrane using the ionophore amphotericin B. This artificially removes all electrical resistance to ion transport across the plasma membrane containing pores formed by amphotericin B. As a result, the intact contralateral plasma membrane becomes rate limiting for transepithelial ion transport. Agonist-dependent changes in ion conductances can be assessed directly either as transepithelial short circuit current (Isc) in the presence of established ion gradients, or as transepithelial conductance (G) in the presence of established transepithelial potentials.

K+ transport was measured at baseline and after the ordered additions of cAMP- and Ca^{++} -agonists. The initial permeabilization with amphotericin B was associated with 49 ± 19% increase in conductance. Pores formed by amphotericin B display selectivity for monovalent cations. Ca^{++} remained relatively impermeant as evidenced by the small steady state increase in Isc and G_K caused by apical permeabilization with amphotericin B. Given this low baseline Isc and G_K, both cAMP- and Ca^{++}-sensitive K+ permeabilities (PK) were readily apparent after agonist stimulation. Treatment with the cAMP-agonist forskolin (10 μM) caused a brisk increase in K+ transport through apparently low-conductance pathway(s), as evidenced by symmetrical increases in Isc and G. Carbachol also increased K+ currents. The magnitude of the carbachol-induced IscK, however, was similar whether carbachol was added alone or after forskolin (111.7 ± 7.4 vs. 180.7 ± 15.7μA/cm^2 respectively. Thus, there was no clear evidence of synergy between cAMP and Ca^{++} mediated K+ pathways, as would be expected in an apically permeabilized cell system. Analagous to our previous findings in intact T84 monolayers, the forskolin-induced changes in Isc were sustained while the effect of carbachol was short-lived. Both Isc_K and G_K returned to baseline values within 5 min after addition of carbachol.

Formal current/voltage (I/V) relations were defined before and after agonist stimulation to confirm that both cAMP- and Ca^{++}-dependent currents were elicited at physiologic membrane potentials. Thapsigargin was used in place of carbacol as a Ca^{++}-agonist in these studies because the K+ transients elicited by thapsigargin achieve steady state conductances of much longer duration, as in intact monolayers. It was found that under conditions of basolaterally directed K+ gradients, both forskolin and thapsigargin activate

macroscopic outwardly rectified (mucosal to serosal) currents at positive transepithelial voltages. Experimental I/V relations obtained after forskolin and thapsigargin stimulation displayed reversal potentials (- 40 mV) that approximated the calculated Nernst-potential (-85 mV calculated as RT/zQo log $[Na]_{out}/[Na]_{in}$). These results are consistent with the activation of distinct cAMP- and Ca^{++}-sensitive basolateral membrane K+ conductances in conjunction with one or more nonspecific transepithelial ion shunts, possibly occurring through intercellular tight junctions or basolateral membrane "leaks."

To confirm that the observed changes in Isc and G represented K+ transport through K+ selective pathways, the effect of forskolin and carbachol on T84 monolayer conductances were examined using buffers containing Na^+ as the sole permeant cation. These studies were performed using an analogous 135 mM basolaterally directed cation (Na^+) gradient. Increases in Isc and G were not detectable in the absence of K+. Thus, the increases in cation conductances induced by agonist stimulation are specific to K+ transport.

Two pharmacologically distinct K+ efflux pathways have been previously identified in intact T84 cells. One pathway participates in the secretary response to cAMP-dependent agonists and displays sensitivity to Ba^{++} salts. The other K+ efflux pathway mediates the response to Ca^{++}-dependent agonists, and is Ba^{++}-insensitive. These findings were confirmed in the permeabolized cell model. The cAMP-sensitive I_K (elicited by treatment with forskolin, 10 μM) was inhibited by greater than 70% by the addition of $BaCl_2$ (3 mM) to basolateral reservoirs. Ba^{++}, however, had no detectable effect on K+ transport induced by the subsequent addition of carbachol (100 μM) to the same monolayers. In contrast, when permeabilized monolayers were treated first with carbachol, the induced Ca^{++} I_K was inhibited by 50% by pretreatment with the scorpion venom charybdotoxin (100 nM). Charybdotoxin, however, had no detectable effect on K+ transport induced by the subsequent addition of forskolin. Thus in permeabilized cells, the differential sensitivity of K+ transport to inhibition by the K+ channel blockers $BaCl_2$ and charybdotoxin paralleled exactly the effect of these channel selective inhibitors on K+ transport in intact cells (measured indirectly as a Cl^- current).

Taken together, these studies define the permeabilized T84 cell model, and provide strong evidence that under the defined conditions both Isc and G represent K+ transport through distinct cAMP- and Ca^{++}-sensitive basolateral K+ channels.

8.3 Clotrimazole and 2-chlorophenyl-bs-phenyl methanol, a structurally related stable metabolite, inhibit K+ transport through both cAMP- and Ca^{++}-dependent K+ channels

We next tested the hypothesis that clotrimazole may inhibit directly basolateral membrane K+ channels in human intestinal T84 cells, as it does in the red cell. clotrimazole significantly inhibited the time course of K+ transport after treatment with the cAMP agonist forskolin (10 μM) and the Ca^{++} agonist carbachol (100 μM). Formal IV relations taken at steady state after cAMP or Ca^{++} stimulation confirm that clotrimazole affected both cAMP- and Ca^{++}- sensitive channels. Nearly identical results were obtained with 2-chlorophenyl-bis-phenyl methanol. clotrimazole and its metabolite 2-chlorophenyl-bis-phenyl methanol inhibit directly both cAMP- and Ca^{++}-sensitive intestinal K+ channels indicating that the ring structure in the absence of the imidazole ring sufficient (and perhaps necessary) for this bioactivity.

8.4. Clotrimazole targets the basolateral rather than the apical surface of T84 cells

Methods. Measurement of Cl^- Conductance of the Apical Plasma Membrane. To examine apical Clconductances, Cl^- was used as the sole permeant ion using identical apical and basolateral buffer solutions. Monolayers were pemeabilized basolaterally by the addition of 100 μM Amphotericin B to the serosal reservoir. Generation of voltage-current curves of channel currents were elicited by 1 sec test potentials from -80 to + 80 mV in 10 mV increments in symmetrical high Choline Cl^- buffers.

Results. Studies were performed to determine whether the primary target of clotrimazole was located on the basoolateral or apical cell surfaces. Most rapid inhibition was achieved by incubation with clotrimazole on both sides of the monolayer. However, basolateral application alone was almost as effective as incubation on both sides. Additionally, the apparent potency of inhibition of clotrimazole at a fixed time point was found to be greater when applied basolaterally than apically. This preferential action of clotrimazole at the basolateral surface of the cell is consistent with the hypothesis that its principal targets are basolateral K+ channels.

To confirm these findings, we examined Cl- transport in T84 cell monolayers permeabilized basolaterally with pores formed by amphotericin B. These studies were performed with Cl- as the only permeant anion, and with symmetrical apical and basolateral Cl- concentrations (142 mM). In monolayers not treated with clotrimazole, the addition of

forskolin (10 μM) to basolateral reservoirs increased Cl- conductances significantly over baseline, presumably via activation of the cystic fibrosis transmembrane regulator (CFTR) Cl-channel. In contrast to the clear inhibitory effects of clotrimazole on basolateral K+ conductances, however, clotrimazole had no detectable effect on either forskolin- or thapsigargin-stimulated Cl-conductances. I/V relations for Cl- transport were nearly identical in monolayers treated or not treated with clotrimazole. These data provide further evidence that clotrimazole inhibits Cl- secretion in intact T84 cell monolayers by affecting specifically basolateral K+ channels. Apical membrane Cl-channels are not inhibited.

Example 9. Clotrimazole inhibits Cl^- secretion *in vivo*.

9.1. Chamber studies using rabbit colonic mucosa.

Methods. 4 male, New Zealand rabbits (2.5 kg) were anesthetized by an intravenous injection of pentobarbital (0.5 ml/kg). A 15 cm length of distal colon was removed and opened longitudinally. External muscle layers were removed by blunt dissection and colonic mucosal preparations were mounted in an Ussing chamber (DCTSYS; Precision Instrument Design, CA; 10.3 cm^2 surface area) and incubated with buffer solution containing (in mM): NaCl 122.0, $CaCl_2$, 2.0; $MgSO_4$,1.3; KCl, 5.0, glucose, 20; $NaHCO_3$, 25.0 (pH when gassed with 95% O2/5 $C0_2$; temperature was maintained at 37°C) with and without clotrimazole (3OμM). The volume of fluid on each side of the mucosa was 7 ml.

Potential difference and Isc were monitored continuously and registered every 10 minutes. Luminal and serosal buffer solutions were interfaced via Ag-AgCl electrodes (Voltage/Current Clamp, Model VCC600, Physiologic Instruments, Inc., San Diego, CA, USA) and Ringer/agar bridge to voltage clamp device (model DVC-1000; Voltage/Current Clamp, World Precision Instruments, Inc.). Resistance (R) was calculated using Ohm's law and the Isc and is given in Ω x cm2. After stable baseline resistance and Isc values had been obtained, mucosal preparations were incubated in the presence or absence of serosal clotrimazole (30 μM) for 30 min, and then stimulated by the addition of forskolin (10 μM) or carbachol (10 μM) to the serosal reservoir.

Results. To test the ability of clotrimazole to block K+ channels and thus Cl- secretion in native intestinal tissue, we mounted isolated preparations of rabbit colonic mucosa in Ussing chambers containing modified Ringer's solution with or without clotrimazole (30 μM). After Isc had stabilized, successive additions of forskolin (lO μM) and then cubachol (100

μM) were applied to serosal reservoirs, and Isc and G were monitored continuously. clotrimazole inhibited strongly the time course of forskolin induced Isc. Carbachol had no further effect on Isc in this system.

9.2. Murine model of secretotory diarrhea.

Methods. Treated and control, untreated, mice were gavage fed either clotrimazole (150 mg/kg/day divided in two equal doses, dissolved in peanut oil at a concentration of 20 mg/ml) or vehicle control over a 7 day loading period. Mice were then challenged by gavage with either 25 μg purified cholera toxin (Calbiochem, San Diego, CA) in PBS, vehicle control alone (PBS without cholera toxin), or cholera toxin in PBS containing 30 μM clotrimazole. Animals were sacrificed after 5 hours in an uncrowded $C0_2$ hood. The carcass was weighed, the abdomen was opened, and ligatures were tied at the proximal duodenum and distal rectum. The intestinal block was dissected free of supporting structures and removed as a single unit and weighed. Small and large intestinal segments were normalized to body weight (intestinal weight/carcass weight) for each animal.

Results. To examine whether clotrimazole may inhibit intestinal secretion in vivo, we utilized a murine model of secretary diarrhea. Balb/C mice were gavage fed 150 mg/kg/day clotrimazole, divided into two equal doses, or vehicle control every 12 h for 7 days and subsequently challenged orally with purified cholera toxin (25 μg). Five hours after treatment with cholera toxin, the mice were sacrificed and intestinal fluid secretion assessed gravimetrically. Pretreatment with clotrimazole reduced by 86% intestinal fluid secretion induced by cholera toxin. Clotrimazole had no effect on intestinal secretion in the absence of cholera toxin. Thus, clotrimazole effectively treated secretory diarrhea in vivo, presumably by inhibiting basolateral K+ channels of crypt epithelial cells.

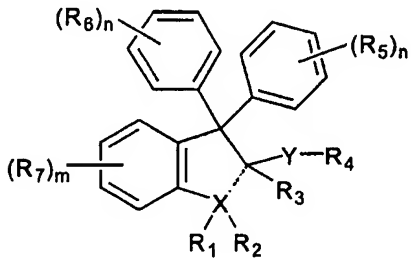
The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

Each of the foregoing patents, patent applications and references is herein incorporated by reference in its entirety.

What Is Claimed Is:

Claims

1. A method for inhibiting unwanted cellular proliferation associated with an inflammatory disease, said method comprising the step of contacting a cell the proliferation of which contributes to inflammation *in situ* with an effective amount of a compound having the formula:



(I)

or a pharmaceutically acceptable salt or hydrate thereof, wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', -NR'_2, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -$C(O)NR'_2$ or -$C(S)NR'_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'_2, -ONR'_2, -SNR'_2, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -$C(O)NR'_2$, -$C(S)NR'_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -$CH(CN)_2$, -$CH[C(O)R']_2$, -$CH[C(S)R']_2$, -$CH[C(O)OR']_2$, -$CH[C(S)OR']_2$, -$CH[C(O)SR']_2$ and -$CH[C(S)SR']_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -C(O)NR'_2, -C(S)NR'_2, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'_2, -C(S)NR'_2 and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and

--- designates a single or double bond,

provided that the unwanted cellular proliferation associated with an inflammatory disease is not a disease selected from the group consisting of cancer, actinic keratosis, and Kaposi's sarcoma.

2. The method of Claim 1, wherein in the compound of structural formula (I):

m is 0 or 1;

each n is independently 0 or 1;

X is C or N;

Y is absent, (C_1-C_3) alkyl, (C_1-C_3) alkenyl or (C_1-C_3) alkynyl;

R_1 is absent -H, -OR, =O, -NR_2, =N-OR, -O-C(O)R, or when taken together with R_2 is 3-5 membered oxirane or 3-5 membered substituted oxirane;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR, -NR_2, -CN, -C(O)OR, -C(O)NR_2 or 5-6 membered dioxoycycloalkyl;

each R_5, R_6 and R_7 is independently selected from the group consisting of -R', -F, -Cl or -Br;

each R is independently selected from the group consisting of -H, (C_1-C_3) alkyl, (C_1-C_3) alkenyl, (C_1-C_3) alkynyl, (C_5-C_{10}) aryl, substituted (C_5-C_{10}) aryl, (C_6-C_{13}) alkaryl, substituted C_6-C_{13}) alkaryl;

the oxirane substituent is -CN, -NO_2, -NR'_2, -OR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of -F, -Cl, -Br, -CN, -NO_2, -NR'_2, -C(O)R', -C(O)OR' and trihalomethyl;

R' is -H, (C_1-C_3) alkyl, (C_1-C_3) alkenyl or (C_1-C_3) alkynyl; and

--- is a single or double bond.

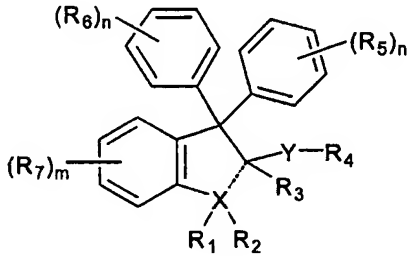
3. The method of Claim 2, wherein said compound is selected from the group consisting of Compounds **1, 2, 3, 4, 7, 9, 12, 13, 14** and combinations thereof.

4. The method of Claim 1, wherein said administration is selected from the group consisting of oral, parenteral, intravenous, subcutaneous, transdermal and transmucosal for a living human.

5. The method of Claim 1, wherein said mammalian cell is a fibrotic cell .

6. The method of Claim 1, wherein said mammalian cell is a lymphocyte.

7. A method of treating or preventing an inflammatory disease, said method comprising the step of administering to a subject suffering from an inflammatory disease a therapeutically effective amount of a compound having the formula:



(I)

or a pharmaceutically acceptable salt or hydrate thereof, wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', -NR'$_2$, -CN, -NO$_2$, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'$_2$ or -C(S)NR'$_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'$_2$, -ONR'$_2$, -SNR'$_2$, -NO$_2$, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -CH(CN)$_2$, -CH[C(O)R']$_2$, -CH[C(S)R']$_2$, -CH[C(O)OR']$_2$, -CH[C(S)OR']$_2$, -CH[C(O)SR']$_2$ and -CH[C(S)SR']$_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO$_2$, -NR'$_2$, -OR', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'$_2$, -C(S)NR'$_2$ and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and

--- designates a single or double bond,

provided that the inflammatory disease is not a disease selected from the group consisting of cancer, actinic keratosis, and Kaposi's sarcoma.

8. The method of Claim 7, wherein in the compound of structural formula (I):

m is 0 or 1;

each n is independently 0 or 1;

X is C or N;

Y is absent, (C_1-C_3) alkyl, (C_1-C_3) alkenyl or (C_1-C_3) alkynyl;

R_1 is absent -H, -OR, =O, -NR_2, =N-OR, -O-C(O)R, or when taken together with R_2 is 3-5 membered oxirane or 3-5 membered substituted oxirane;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR, -NR_2, -CN, -C(O)OR, -C(O)NR_2 or 5-6 membered dioxoycycloalkyl;

each R_5, R_6 and R_7 is independently selected from the group consisting of -R', -F, -Cl or -Br;

each R is independently selected from the group consisting of -H, (C_1-C_3) alkyl, (C_1-C_3) alkenyl, (C_1-C_3) alkynyl, (C_5-C_{10}) aryl, substituted (C_5-C_{10}) aryl, (C_6-C_{13}) alkaryl, substituted C_6-C_{13}) alkaryl;

the oxirane substituent is -CN, -NO_2, -NR'_2, -OR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of -F, -Cl, -Br, -CN, -NO_2, -NR'_2, -C(O)R', -C(O)OR' and trihalomethyl;

R' is -H, (C_1-C_3) alkyl, (C_1-C_3) alkenyl or (C_1-C_3) alkynyl; and

--- is a single or double bond.

9. The method of Claim 8, wherein said compound is selected from the group consisting of Compounds **1**, **2**, **3**, **4**, **7**, **9**, **12**, **13**, **14**, and combinations thereof.

10. The method of Claim 7, wherein said inflammatory disease is diarrhea.

11. The method of Claim 10, wherein said diarhrea is caused by inflammatory bowel disease.

12. The method of Claim 7, wherein said inflammatory disease is an autoimmune disease.

13. The method of Claim 12, wherein said autoimmune disease is lupus.

14. The method of Claim 7, wherein said inflammatory disease is glomerulonephritis.

15. The method of Claim 7, wherein said administration is parenteral.

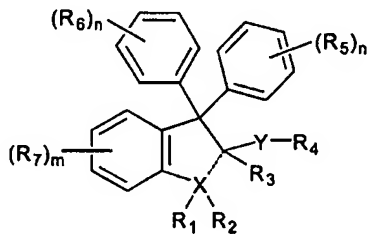
16. The method of Claim 7, wherein said administration is per oral.

17. The method of claim 7, wherein the inflammatory disease is selected from the group consisting of proliferative glomerulonephritis; lupus erythematosus; scleroderma; temporal arteritis; thromboangiitis obliterans; mucocutaneous lymph node syndrome; asthma; host versus graft; inflammatory bowel disease; multiple sclerosis; rheumatoid arthritis; thyroiditis; Grave's disease; antigen-induced airway hyperactivity; pulmonary eosinophilia; Guillain-Barre syndrome; allergic rhinitis; myasthenia gravis; human T-lymphotrophic virus type 1-associated myelopathy; herpes simplex encephalitis; inflammatory myopathies; atherosclerosis; and Goodpasture's syndrome.

18. A method for treating diarrhea, comprising, administering an effective amount for inhibiting Cl^- secretion of an aromatic compound to a subject, wherein the aromatic compound is selected from the group consisting of a substituted or unsubstituted 3,3-diphenyl indanone, a substituted or unsubstituted indane, a substituted or unsubstituted (*3-H*) indole compound, and analogues of these classes of compounds wherein the atoms at ring positions 1 and 2 are connected via a double bond.

19. The method of claim 18, wherein the aromatic compound is a compound having the structural formula:

(I)



or pharmaceutically acceptable salts or hydrates thereof, wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', -NR'$_2$, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'$_2$ or -C(S)NR'$_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'$_2$, -ONR'$_2$, -SNR'$_2$, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -CH(CN)$_2$, -CH[C(O)R']$_2$, -CH[C(S)R']$_2$, -CH[C(O)OR']$_2$, -CH[C(S)OR']$_2$, -CH[C(O)SR']$_2$ and -CH[C(S)SR']$_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'$_2$, -OR', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'$_2$, -C(S)NR'$_2$ and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and

--- designates a single or double bond.

20. The method of claim 18, wherein the aromatic compound is selected from the group consisting of aromatic compounds wherein m is 0, 1, 2, 3 or 4; each n is independently 0, 1, 2, 3, 4 or 5; X is C or N; Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl; R_1 is absent, -OR, =O, =N-OR, -O-C(O)R, or when taken together with R_2 is a 3-8 membered

oxirane or a substituted 3-8 membered oxirane; R_2 is absent or -H; R_3 is absent or -H; R_4 is -H, -OR', -NR'_2, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered oxiranyl, 5-8 membered dioxycycloalkyl, -C(O)R', -C(O)OR' or -$C(O)NR'_2$; each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -NR'_2, -ONR'_2, -NO_2, -CN, -C(O)R', -C(O)OR', -$C(O)NR'_2$, -C(O)NR'(OR'), -$CH(CN)_2$, -$CH[C(O)R']_2$ and -$CH[C(O)OR']_2$; each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl; the oxirane substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -$C(O)NR'_2$, -C(O)OR' and trihalomethyl; the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(O)OR', -$C(O)NR'_2$ and trihalomethyl; each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and/or --- designates a single or double bond.

21. The method of claim 19, wherein the aromatic compound is administered orally.

22. The method of claim 19, wherein the subject is a human.

23. The method of claim 22, further comprising administering an anti-diarrheal agent to the subject.

24. The method of claim 23, wherein the anti-diarrheal agent is an oral rehydration fluid.

25. The method of claim 19, wherein the aromatic compound is selected from the group consisting of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

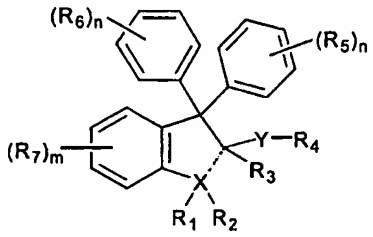
26. A veterinary preparation comprising:

an aromatic compound in an amount effective to inhibit scours in a subject, wherein the aromatic compound is selected from the group consisting of a substituted or unsubstituted 3,3-diphenyl indanone, a substituted or unsubstituted indane, a substituted or unsubstituted (*3*-

H) indole compound, and analogues of these classes of compounds wherein the atoms at ring positions 1 and 2 are connected via a double bond; and,

an anti-scours agent.

27. A veterinary preparation as in claim 26, wherein the aromatic compound is a compound having the structural formula:

(I) 

or pharmaceutically acceptable salts or hydrates thereof, wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', -NR'$_2$, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'$_2$ or -C(S)NR'$_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'$_2$, -ONR'$_2$, -SNR'$_2$, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -$CH(CN)_2$, -CH[C(O)R']$_2$, -CH[C(S)R']$_2$, -CH[C(O)OR']$_2$, -CH[C(S)OR']$_2$, -CH[C(O)SR']$_2$ and -CH[C(S)SR']$_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl,

(C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -C(O)NR'_2, -C(S)NR'_2, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'_2, -C(S)NR'_2 and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and

--- designates a single or double bond.

28. The veterinary preparation as in claim 26, wherein the anti-scours agent is a colostral extract.

29. The veterinary preparation as in claim 26, wherein the anti-scours agent is an immunological preparation of colostrum.

30. The veterinary preparation as in claim 26, wherein the anti-scours agent is a microorganism specific immunological preparation.

31. The veterinary preparation as in claim 26, wherein the anti-scours agent is an oral rehydration fluid.

32. The veterinary preparation as in claim 26, wherein the anti-scours agent is a replacement electrolyte composition.

33. The veterinary preparation as in claim 26, wherein the anti-scours agent is an antibiotic composition.

34. The veterinary preparation as in claim 26, wherein the veterinary preparation is

a dry preparation.

35. The veterinary preparation as in claim 26, wherein the aromatic compound is selected from the group consisting of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

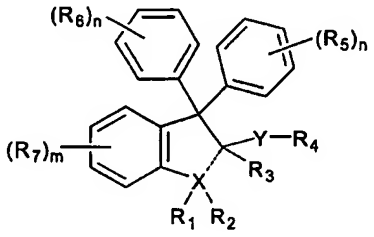
36. A pharmaceutical preparation, comprising:

an aromatic compound in an amount effective to inhibit diarrhea,wherein the aromatic compound is selected from the group consisting of a substituted or unsubstituted 3,3-diphenyl indanone, a substituted or unsubstituted indane, a substituted or unsubstituted (*3-H*) indole compound, and analogues of these classes of compounds wherein the atoms at ring positions 1 and 2 are connected via a double bond; and,

an anti-diarrheal agent.

37. The pharmaceutical preparation as in claim 36, wherein the aromatic compound is a compound having the structural formula:

(I)



or pharmaceutically acceptable salts or hydrates thereof, wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', -NR'$_2$, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'$_2$ or -C(S)NR'$_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'$_2$, -ONR'$_2$, -SNR'$_2$, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -CH(CN)$_2$, -CH[C(O)R']$_2$, -CH[C(S)R']$_2$, -CH[C(O)OR']$_2$, -CH[C(S)OR']$_2$, -CH[C(O)SR']$_2$ and -CH[C(S)SR']$_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'$_2$, -OR', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'$_2$, -C(S)NR'$_2$ and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and

--- designates a single or double bond.

38. The pharmaceutical preparation as in claim 37, wherein the aromatic compound is selected from the group consisting of aromatic compounds wherein m is 0, 1, 2, 3 or 4; each n is independently 0, 1, 2, 3, 4 or 5; X is C or N; Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl; R_1 is absent, -OR, =O, =N-OR, -O-C(O)R, or when taken together with R_2 is a 3-8 membered oxirane or a substituted 3-8 membered oxirane; R_2 is absent or -H; R_3 is absent or -H; R_4 is -H, -OR', -NR'$_2$, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered oxiranyl, 5-8 membered dioxycycloalkyl, -C(O)R', -C(O)OR' or -C(O)NR'$_2$; each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -NR'$_2$, -ONR'$_2$, -NO_2, -CN, -C(O)R', -C(O)OR', -C(O)NR'$_2$, -C(O)NR'(OR'), -CH(CN)$_2$, -CH[C(O)R']$_2$ and -CH[C(O)OR']$_2$; each R is independently selected from the group

consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl; the oxirane substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -C(O)NR'_2, -C(O)OR' and trihalomethyl; the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(O)OR', -C(O)NR'_2 and trihalomethyl; each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and/or --- designates a single or double bond.

39. The pharmaceutical preparation as in claim 37, wherein the anti-diarrheal agent is an oral rehydration fluid.

40. The pharmaceutical preparation as in claim 37, wherein the anti-diarrheal agent is an antibiotic.

41. The pharmaceutical preparation as in claim 37, wherein the anti-diarrheal agent is an electrolyte composition.

42. The pharmaceutical preparation as in claim 37, wherein the anti-diarrheal agent is an immunoglobulin preparation from bovine colostrum.

43. The pharmaceutical preparation as in claim 37, wherein the anti-diarrheal agent is an oral sugar-electrolyte solution.

44. The pharmaceutical preparation as in claim 37, wherein the aromatic compound is selected from the group consisting of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

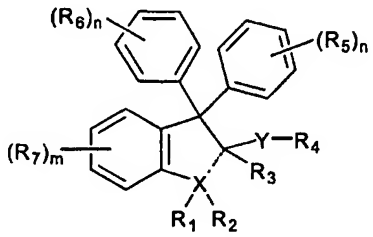
45. A method for treating scours, the method comprising the step of:

administering to a subject in need of such treatment, an aromatic compound in an amount effective to inhibit scours,wherein the aromatic compound is selected from the group consisting of a substituted or unsubstituted 3,3-diphenyl indanone, a substituted or unsubstituted indane, a substituted or unsubstituted (*3-H*) indole compound, and analogues of

these classes of compounds wherein the atoms at ring positions 1 and 2 are connected via a double bond.

46. The method for treating scours as in claim 45, wherein the aromatic compound is a compound having the structural formula:

(I)



or pharmaceutically acceptable salts or hydrates thereof, wherein:

m is 0, 1, 2, 3 or 4;

each n is independently 0, 1, 2, 3, 4 or 5;

X is C or N;

Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl;

R_1 is absent, -OR, -SR, =O, =S, =N-OR, -O-C(O)R, -S-C(O)R, -O-C(S)R, -S-C(S)R, or when taken together with R_2 is a 3-8 membered heterocycloalkyl or a substituted 3-8 membered heterocycloalkyl;

R_2 is absent or -H;

R_3 is absent or -H;

R_4 is -H, -OR', -SR', -NR'$_2$, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered heterocycloalkyl, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'$_2$ or -C(S)NR'$_2$;

each R_5, R_6 and R_7 is independently selected from the group consisting of -halogen, -R', -OR', -SR', -NR'$_2$, -ONR'$_2$, -SNR'$_2$, -NO_2, -CN, -C(O)R', -C(S)R', -C(O)OR', -C(O)SR', -C(S)OR', -CS(S)R', -C(O)NR'$_2$, -C(S)NR'$_2$, -C(O)NR'(OR'), -C(S)NR'(OR'); -C(O)NR'(SR'), -C(S)NR'(SR'), -CH(CN)$_2$, -CH[C(O)R']$_2$, -CH[C(S)R']$_2$, -CH[C(O)OR']$_2$, -CH[C(S)OR']$_2$, -CH[C(O)SR']$_2$ and -CH[C(S)SR']$_2$;

each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and

substituted (C_6-C_{26}) alkaryl;

the heterocycloalkyl substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -C(O)NR'_2, -C(S)NR'_2, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR' and trihalomethyl;

the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(S)R', -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', -C(O)NR'_2, -C(S)NR'_2 and trihalomethyl;

each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and

--- designates a single or double bond.

47. The method for treating scours as in claim 46, wherein the aromatic compound is selected from the group consisting of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

48. The method for treating scours as in claim 46, wherein the aromatic compound is administered orally.

49. The method for treating scours as in claim 46, wherein the subject is selected from the group consisting of a horse, a cow, a pig, and a goat.

50. The method for treating scours as in claim 46, further comprising administering an anti-scours agent to the subject.

51. The method for treating scours as in claim 46, wherein the aromatic compound is selected from the group consisting of aromatic compounds wherein m is 0, 1, 2, 3 or 4; each n is independently 0, 1, 2, 3, 4 or 5; X is C or N; Y is absent, (C_1-C_6) alkyl, (C_1-C_6) alkenyl or (C_1-C_6) alkynyl; R_1 is absent, -OR, =O, =N-OR, -O-C(O)R, or when taken together with R_2 is a 3-8 membered oxirane or a substituted 3-8 membered oxirane; R_2 is absent or -H; R_3 is absent or -H; R_4 is -H, -OR', -NR'_2, -CN, -NO_2, (C_3-C_8) cycloalkyl, 3-8 membered oxiranyl, 5-8 membered dioxycycloalkyl, -C(O)R', -C(O)OR' or -C(O)NR'_2; each R_5, R_6 and R_7 is

independently selected from the group consisting of -halogen, -R', -OR', -NR'_2, -ONR'_2, -NO_2, -CN, -C(O)R', -C(O)OR', -$C(O)NR'_2$, -C(O)NR'(OR'), -$CH(CN)_2$, -$CH[C(O)R']_2$ and -$CH[C(O)OR']_2$; each R is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl, (C_1-C_6) alkynyl, (C_5-C_{20}) aryl, substituted (C_5-C_{20}) aryl, (C_6-C_{26}) alkaryl and substituted (C_6-C_{26}) alkaryl; the oxirane substituents are each independently selected from the group consisting of -CN, -NO_2, -NR'_2, -OR', -$C(O)NR'_2$, -C(O)OR' and trihalomethyl; the aryl and alkaryl substituents are each independently selected from the group consisting of halogen, -C(O)R', -C(O)OR', -$C(O)NR'_2$ and trihalomethyl; each R' is independently selected from the group consisting of -H, (C_1-C_6) alkyl, (C_1-C_6) alkenyl and (C_1-C_6) alkynyl; and/or --- designates a single or double bond.

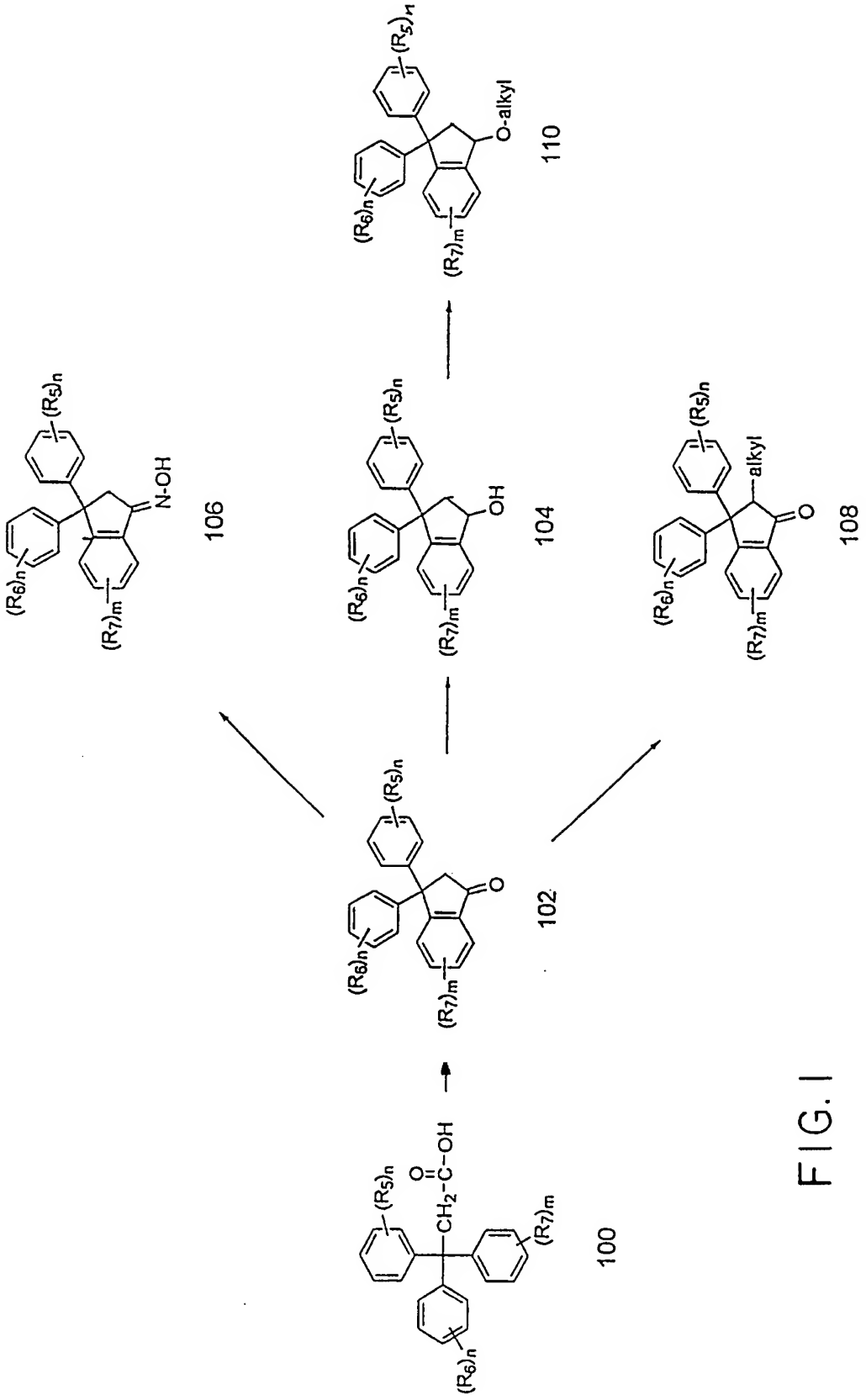

(R5)n
O
CH2-C-OH
(R7)m
(R6)n
100
102
104
106
108
110
N-OH
OH
alkyl
O-alkyl

FIG. 1

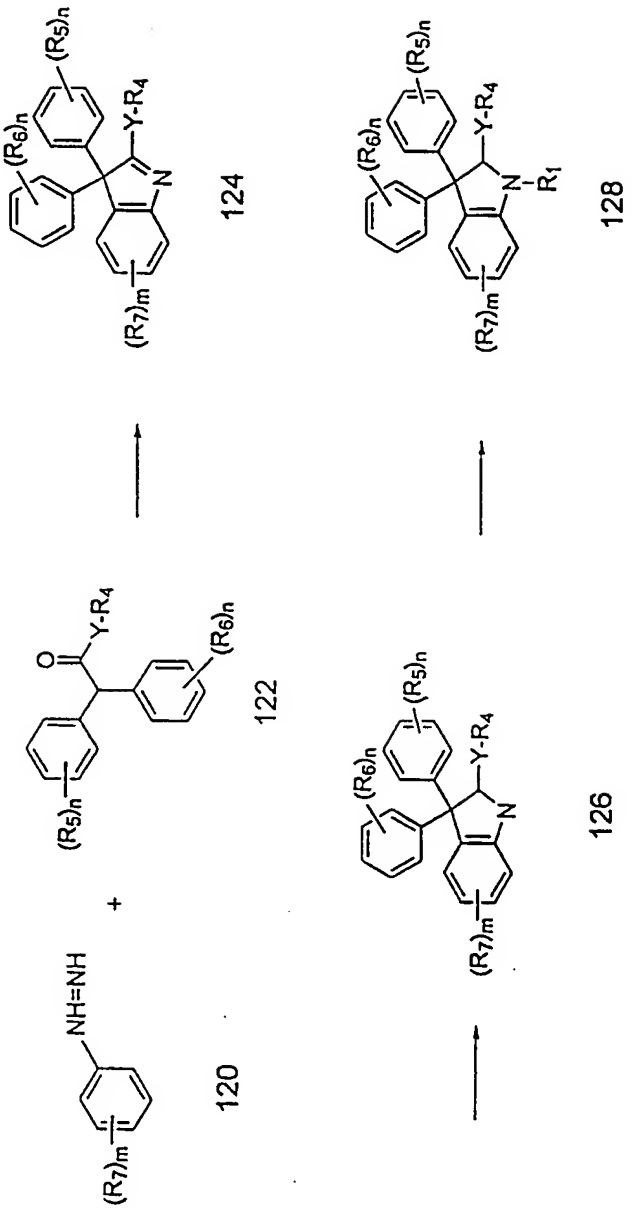

NH=NH
(R7)m
120
+
(R5)n
(R6)n
O
Y-R4
122
(R7)m
(R5)n
(R6)n
Y-R4
N
124
(R7)m
(R5)n
(R6)n
Y-R4
N
R1
128
(R7)m
(R5)n
(R6)n
Y-R4
N
126

FIG. 2

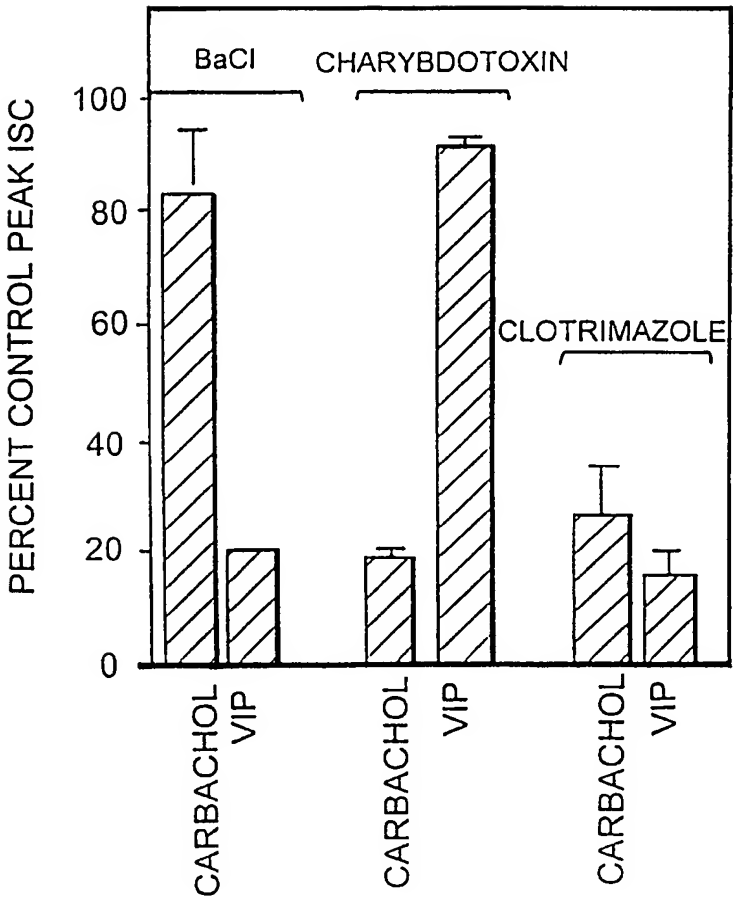

BaCl
CHARYBDOTOXIN
CLOTRIMAZOLE
PERCENT CONTROL PEAK ISC
100
80
60
40
20
0
CARBACHOL
VIP
CARBACHOL
VIP
CARBACHOL
VIP

FIG. 3

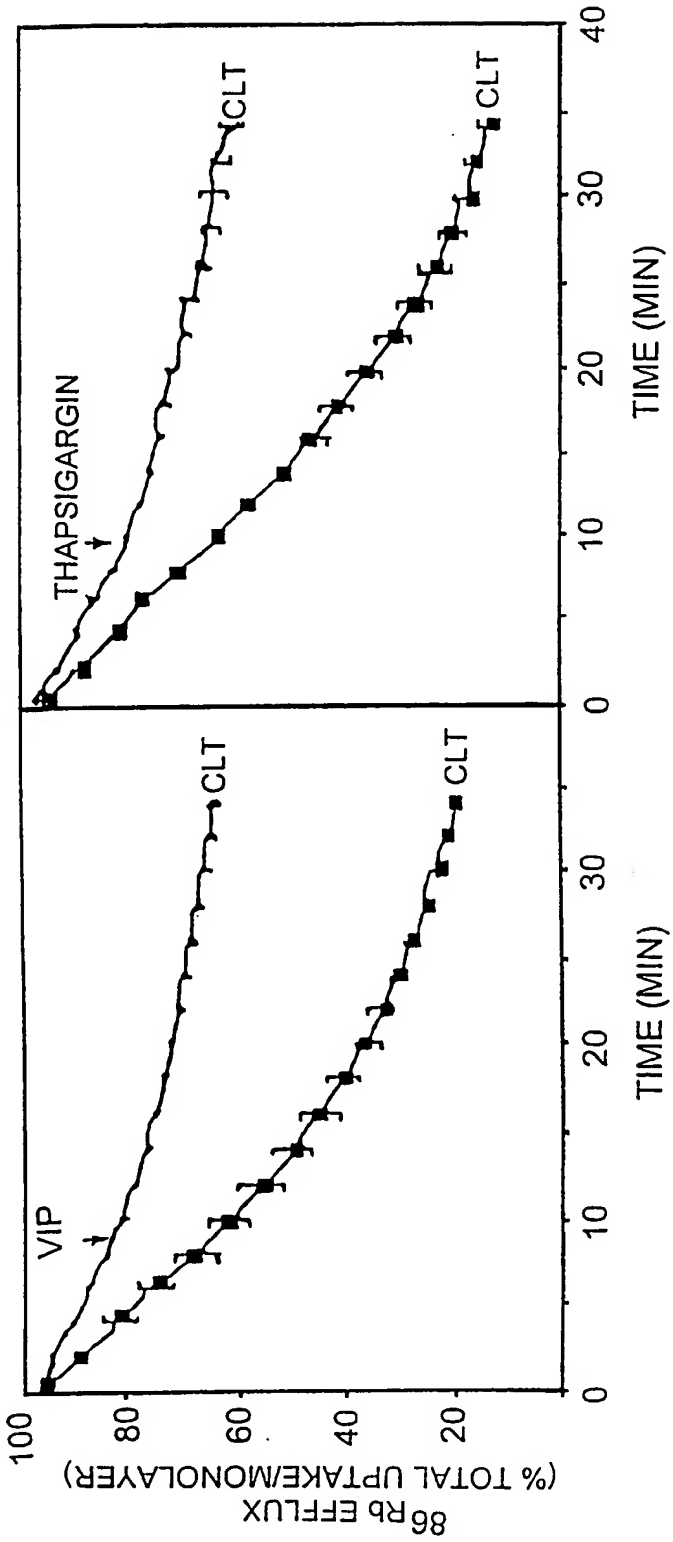

^{86}Rb EFFLUX
(% TOTAL UPTAKE/MONOLAYER)
100
80
60
40
20
0
10
20
30
40
TIME (MIN)
VIP
CLT
CLT
THAPSIGARGIN
CLT
CLT
TIME (MIN)

FIG. 4

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 98/24968

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/40 A61K31/015 C07C13/465

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 12613 A (WARNER-LAMBERT COMPANY) 10 April 1997 see claims 1,23,24 ---	1-51
A	WO 96 08242 A (CHILDREN'S MEDICAL CENTER CORPORATION) 21 March 1996 see claims 1-24 see page 8, line 6 - line 12 ---	1-51
A	US 5 273 992 A (C. BRUGNARA ET AL) 28 December 1993 cited in the application see the whole document ---	1-51
A	WO 97 34589 A (PRESIDENT AND FELLOWS OF HARVARD UNIVERSITY ET AL) 25 September 1997 see claims 1-42 -----	1-51

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 April 1999	16/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Siatou, E

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/24968

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1-25, 45-51 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter ...nal Application No
PCT/US 98/24968

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9712613 A	10-04-1997	AU 6966696 A	28-04-1997
		AU 7254196 A	28-04-1997
		WO 9712615 A	10-04-1997
WO 9608242 A	21-03-1996	AU 3634795 A	29-03-1996
		EP 0781128 A	02-07-1997
US 5273992 A	28-12-1993	US 5441957 A	15-08-1995
WO 9734589 A	25-09-1997	AU 2538897 A	10-10-1997